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(54) Title: INHIBITORS OF HISTONE DEACETYLASE

(57) Abstract: The invention provides compoods and methods for treating cell proliferative- diseases. The invention provides new inhibitors of histone deacetylase enzymatic activity, compositions of the compounds comprising the inhibitors and a pharmaceutically acceptable carrier, excipient, or diluent, and methods of using the compounds to inhibit cellular proliferation in vitro and therapeutically.

INHIBITORS OF HISTONE DEACETYLASE

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates to the inhibition of histone deacetylase. More particularly, the invention relates to compounds and methods for inhibiting histone deacetylase enzymatic activity.

Summary of the Related Art

[0002] In eukaryotic cells, nuclear DNA associates with histones to form a compact complex called chromatin. The histones constitute a family of basic proteins which are generally highly conserved across eukaryotic species. The core histones, termed H2A, H2B, H3, and H4, associate to form a protein core. DNA winds around this protein core, with the basic amino acids of the histones interacting with the negatively charged phosphate groups of the DNA. Approximately 146 base pairs of DNA wrap around a histone core to make up a nucleosome particle, the repeating structural motif of chromatin.

[0003] Csordas, *Biochem. J.*, **286**: 23-38 (1990) teaches that histones are subject to posttranslational acetylation of the α,ε-amino groups of *N*-terminal lysine residues, a reaction that is catalyzed by histone acetyl transferase (HAT1). Acetylation neutralizes the positive charge of the lysine side chain, and is thought to impact chromatin structure. Indeed, Taunton *et al.*, *Science*, **272**: 408-411 (1996), teaches that access of transcription factors to chromatin templates is enhanced by histone hyperacetylation. Taunton *et al.* further teaches that an enrichment in underacetylated histone H4 has been found in transcriptionally silent regions of the genome.

[0004] Histone acetylation is a reversible modification, with deacetylation being catalyzed by a family of enzymes termed histone deacetylases (HDACs). Grozinger *et al.*, *Proc. Natl. Acad. Sci. USA*, **96**: 4868-4873 (1999), teaches that HDACs is divided into two classes, the first represented by yeast Rpd3-like proteins, and the second represented by yeast Hda1-like proteins. Grozinger *et al.* also teaches that the human HDAC1, HDAC2, and HDAC3 proteins are members of the first class of HDACs, and discloses new proteins, named HDAC4, HDAC5, and HDAC6, which are members of the second class of HDACs. Kao *et al.*, *Genes & Dev.*, **14**: 55-66 (2000), discloses HDAC7, a new member of the second class of HDACs. Van den Wyngaert, *FEBS*, **478**: 77-83 (2000) discloses HDAC8, a new member of the first class of HDACs.

[0005] Richon *et al.*, *Proc. Natl. Acad. Sci. USA*, **95**: 3003-3007 (1998), discloses that HDAC activity is inhibited by trichostatin A (TSA), a natural product isolated from *Streptomyces*

hygroscopicus, and by a synthetic compound, suberoylanilide hydroxamic acid (SAHA). Yoshida and Beppu, *Exper. Cell Res.*, **177**: 122-131 (1988), teaches that TSA causes arrest of rat fibroblasts at the G_1 and G_2 phases of the cell cycle, implicating HDAC in cell cycle regulation. Indeed, Finnin *et al.*, *Nature*, **401**: 188-193 (1999), teaches that TSA and SAHA inhibit cell growth, induce terminal differentiation, and prevent the formation of tumors in mice. Suzuki *et al.*, U.S. Pat. No. 6,174,905, EP 0847992, JP 258863/96, and Japanese Application No. 10138957, disclose benzamide derivatives that induce cell differentiation and inhibit HDAC. Delorme *et al.*, WO 01/38322 and PCT IB01/00683, disclose additional compounds that serve as HDAC inhibitors.

[0006] These findings suggest that inhibition of HDAC activity represents a novel approach for intervening in cell cycle regulation and that HDAC inhibitors have great therapeutic potential in the treatment of cell proliferative diseases or conditions. To date, few inhibitors of histone deacetylase are known in the art. There is thus a need to identify additional HDAC inhibitors and to identify the structural features required for potent HDAC inhibitory activity.

BRIEF SUMMARY OF THE INVENTION

[0007] The invention provides compounds and methods for treating cell proliferative diseases. The invention provides new inhibitors of histone deacetylase enzymatic activity.

[0008] In a first aspect, the invention provides compounds that are useful as inhibitors of histone deacetylase.

[0009] In a second aspect, the invention provides a pharmaceutical composition comprising an inhibitor of histone deacetylase according to the invention and a pharmaceutically acceptable carrier, excipient, or diluent.

[0010] In a third aspect, the invention provides a method of inhibiting histone deacetylase in a cell, comprising contacting a cell in which inhibition of histone deacetylase is desired with an inhibitor of histone deacetylase of the invention.

[0011] The foregoing merely summarizes certain aspects of the invention and is not intended to be limiting in nature. These aspects and other aspects and embodiments are described more fully below.

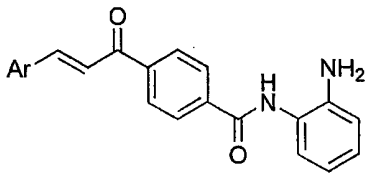
BRIEF DESCRIPTION OF THE DRAWING

[0012] **Figure 1** displays antineoplastic effects of a histone deacetylase inhibitor according to the invention on human tumor xenografts *in vivo*, as described in Example 51, *infra*.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0013] The invention provides compounds and methods for inhibiting histone deacetylase enzymatic activity. The invention also provides compositions and methods for treating cell proliferative diseases and conditions. The patent and scientific literature referred to herein establishes knowledge that is available to those with skill in the art. The issued patents, applications, and references that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of inconsistencies, the present disclosure will prevail.

[0014] In one embodiment of the first aspect, the invention comprises compounds of the following formula:



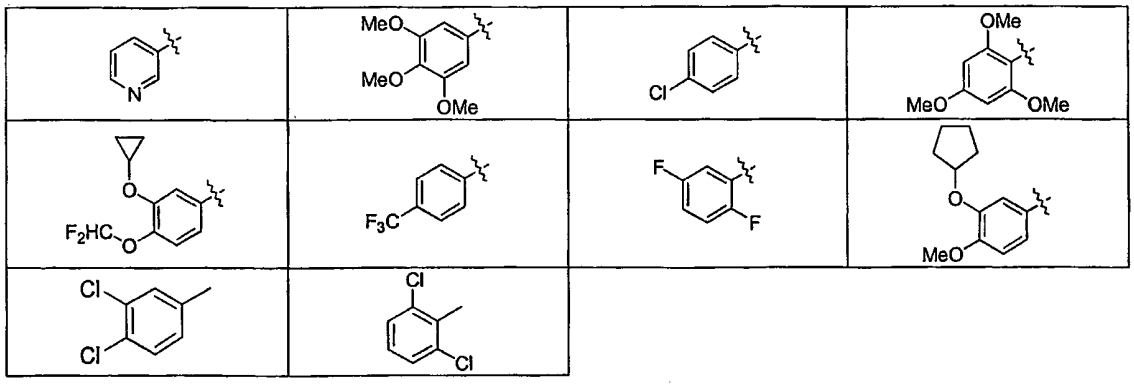
and pharmaceutically acceptable salts thereof, wherein

Ar is aryl or heteroaryl, each of which is optionally substituted with from 1 to 3 substituents.

[0015] Preferably, Ar is aryl or pyridinyl in the compound of paragraph [0014].

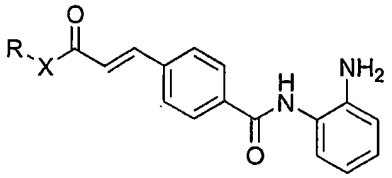
[0016] Preferred substituents of Ar include halo, C_1-C_6-hydrocarbyl optionally substituted with halo, C_1-C_6-hydrocarbyloxy optionally substituted with halo. Particularly preferred substituents include fluoro, chloro, methoxy, cyclopropyloxy, and cyclopentyloxy.

[0017] In a preferred embodiment of the compound according to paragraph [0014], Ar is selected from the following:



[0018] Preferred compounds of paragraph [0014] include those of Table 6 below.

[0019] In another embodiment of the first aspect, the invention comprises compounds of the following formula:



and pharmaceutically acceptable salts thereof, wherein

X is -N(R^1)-, -O-, or -S-; or X is a nitrogen-containing heterocyclyl in which a nitrogen is covalently bound to the adjacent carbonyl in structure V and is optionally substituted with from 1 to 3 substituents; and

R and R^1 independently are -H, or optionally substituted a) C_1-C_6-hydrocarbyl or b) R^2-L-, wherein R^2 is aryl or heteroaryl, L is C_0-C_6-hydrocarbyl-L^1-C_0-C_6-hydrocarbyl, and L^1 is a covalent bond, -O-, -S-, or -NH-.

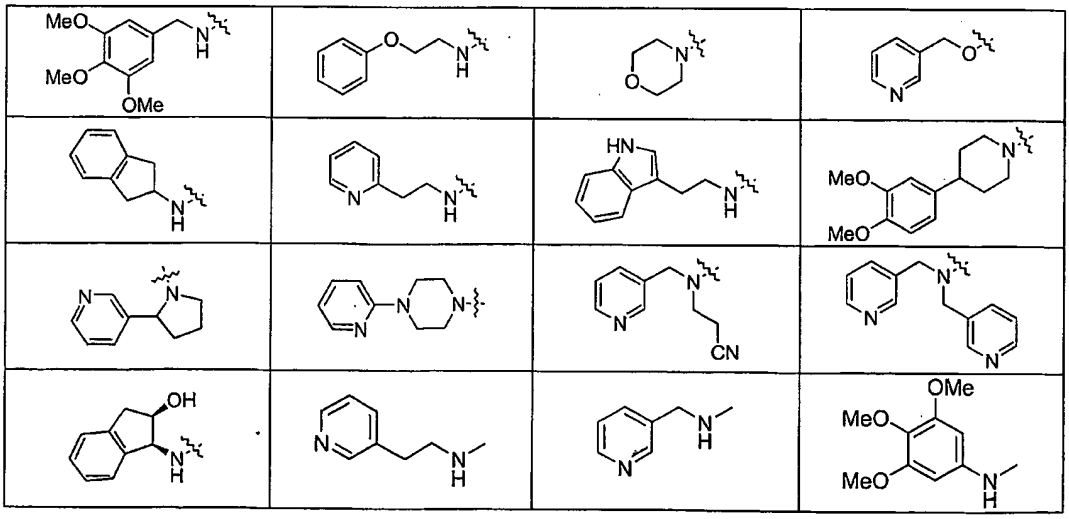
[0020] Preferably in the compound according to paragraph [0019], X is -NH-, -O-, morphilin-4-yl, piperidin-1-yl, piperizin-1-yl, or pyrrolidin-1-yl.

[0021] In another preferred embodiment of the compound according to paragraph [0019], X is -N(R^1)- wherein R^1 is optionally substituted methyl or ethyl. Preferably R^1 is cyanoethyl or pyridinylmethyl.

[0022] Preferably in the compound according to paragraph [0019], R is R^2-L- wherein R^2 is phenyl, pyridinyl, indyl, or indolyl and L is a covalent bond, methyl, ethyl, or oxyethyl.

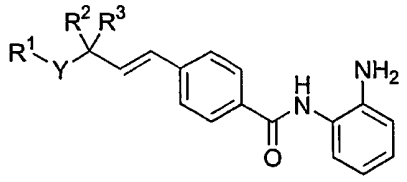
[0023] Preferred substituents of R include methoxy and hydroxy.

[0024] In a preferred embodiment of the compound according to paragraph [0019], the combination of R-X- is selected from the following:



[0025] Preferred compounds according to paragraph [0019] include those listed in Table 7.

[0026] In another embodiment of the first aspect, the invention comprises compounds of the following formula:



and pharmaceutically acceptable salts thereof, wherein

Y is -N(R^4)-, -O-, -S-, -N(R^4)SO_2-, - SO_2-N(R^4) -, -SO_2-, -N(R^4)-C(O)-, -C(O)-N(R^4)-, -NHC(O)NH-, -N(R^4)C(O)O-, -OC(O)N(R^4)-, or a covalent bond, and

R^1, R^2, and R^3 independently are -H or R^a-C_0-C_6-hydrocarbyl wherein R^a is -H or R^a is aryl or heteroaryl, each of which is optionally substituted with from 1 to 3 substituents.

R^4 is -H, -C(O)-R^b, -C(O)O-R^b, -C(O)NH-R^b ,or R^c-C_0-C_6-hydrocarbyl wherein

R^b is -H or -C_1-C_6-hydrocarbyl, and

R^c is -H, or aryl or heteroaryl each of which is optionally substituted with from 1 to 3 substituents.

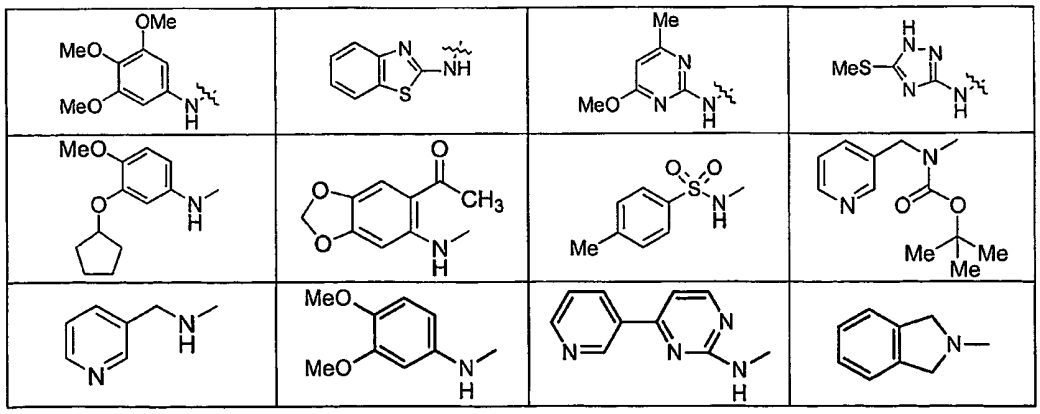
[0027] In the compound of paragraph [0026], R^2 and R^3 are preferably both -H.

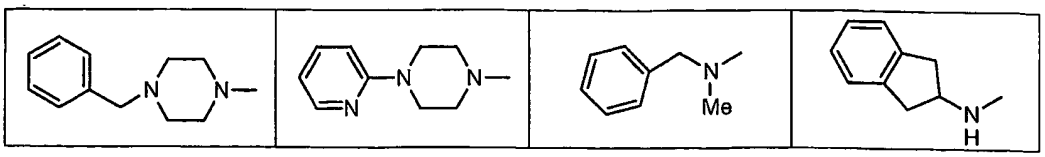
[0028] In the compound of paragraph [0026], Y is preferably -NH-, -SO_2-NH-, or -N(R^4)- wherein R^4 is -C(O)O-C_1-C_6-hydrocarbyl.

[0029] In the compound of paragraph [0026], R^1 is preferably aryl, benzothiazolyl, pyrimidinyl, triazolyl, benzodioxolenyl, or pyridinyl.

[0030] Preferred substituents of R^1 include C_1-C_6-hydrocarbyl, C_1-C_6-hydrocarbyloxy (*e.g.*, methoxy and cyclopropyloxy) halo, methylthio, and acetyl.

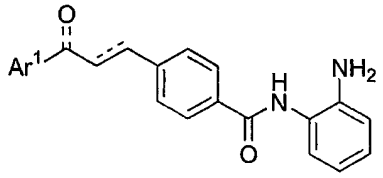
[0031] In a preferred embodiment of the compound according to paragraph [0026], R^1-Y- is selected from the following:





[0032] Preferred compounds according to paragraph [0026] include those listed in Table 8.

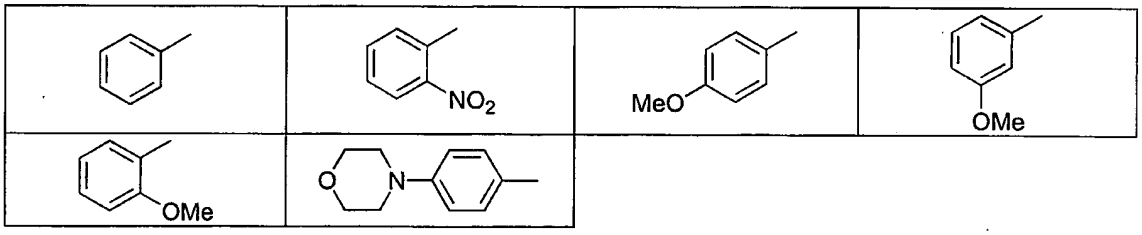
[0033] In another embodiment of the first aspect, the invention comprises compounds of formula:



and pharmaceutically acceptable salts thereof, wherein Ar^1 is aryl or heteroaryl optionally substituted with from 1-3 substituents independently selected from -NO_2, CH_3O-, and morpholinyl (*e.g.*, morpholin-4-yl).

[0034] In a preferred embodiment of the compound according to paragraph [0033], Ar^1 is aryl (*e.g.*, phenyl).

[0035] In preferred embodiments of the compound according to paragraph [0033], Ar^1 is selected from:



[0036] Preferred compounds according to paragraph [0033] included those listed in Table 9.

[0037] In the second aspect, the invention comprises a composition comprising a compound according to one of paragraphs [0014]-[0036] and a pharmaceutically acceptable carrier, excipient, or diluent.

[0038] In a third aspect, the invention provides a method of inhibiting histone deacetylase in a cell, comprising contacting a cell in which inhibition of histone deacetylase is desired with an inhibitor of histone deacetylase according to one of paragraphs [0014]-[0037].

[0039] In another aspect, the invention comprises treating a mammal (preferably a human) suffering from a cell proliferative diseases or conditions a therapeutically effective amount of a composition according to paragraph [0037].

[0040] For purposes of the present invention, the following definitions will be used (unless expressly stated otherwise):

[0041] As used herein, the terms "histone deacetylase" and "HDAC" are intended to refer to any one of a family of enzymes that remove acetyl groups from the ,-amino groups of lysine residues at the N-terminus of a histone. Unless otherwise indicated by context, the term "histone" is meant to refer to any histone protein, including H1, H2A, H2B, H3, H4, and H5, from any species. Preferred histone deacetylases include class I and class II enzymes. Preferably the histone deacetylase is a human HDAC, including, but not limited to, HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, and HDAC-8. In some other preferred embodiments, the histone deacetylase is derived from a protozoal or fungal source.

[0042] The terms "histone deacetylase inhibitor" and "inhibitor of histone deacetylase" are used to identify a compound having a structure as defined herein, which is capable of interacting with a histone deacetylase and inhibiting its enzymatic activity. "Inhibiting histone deacetylase enzymatic activity" means reducing the ability of a histone deacetylase to remove an acetyl group from a histone. In some preferred embodiments, such reduction of histone deacetylase activity is at least about 50%, more preferably at least about 75%, and still more preferably at least about 90%. In other preferred embodiments, histone deacetylase activity is reduced by at least 95% and more preferably by at least 99%.

[0043] Preferably, such inhibition is specific, *i.e.*, the histone deacetylase inhibitor reduces the ability of a histone deacetylase to remove an acetyl group from a histone at a concentration that is lower than the concentration of the inhibitor that is required to produce another, unrelated biological effect. Preferably, the concentration of the inhibitor required for histone deacetylase inhibitory activity is at least 2-fold lower, more preferably at least 5-fold lower, even more preferably at least 10-fold lower, and most preferably at least 20-fold lower than the concentration required to produce an unrelated biological effect.

[0044] For simplicity, chemical moieties are defined and referred to throughout primarily as univalent chemical moieties (*e.g.*, alkyl, aryl, etc.). Nevertheless, such terms are also used to convey corresponding multivalent moieties under the appropriate structural circumstances clear to those skilled in the art. For example, while an "alkyl" moiety generally refers to a monovalent radical (*e.g.* CH_3-CH_2-), in certain circumstances a bivalent linking moiety can be "alkyl," in which case those skilled in the art will understand the alkyl to be a divalent radical (*e.g.*, -CH_2-CH_2-), which is equivalent to the term "alkylene." (Similarly, in circumstances in which a divalent moiety is required and is stated as being "aryl," those skilled in the art will understand that the term "aryl"

refers to the corresponding divalent moiety, arylene.) All atoms are understood to have their normal number of valences for bond formation (*i.e.*, 4 for carbon, 3 for N, 2 for O, and 2, 4, or 6 for S, depending on the oxidation state of the S). On occasion a moiety may be defined, for example, as $(A)_a$-B-, wherein a is 0 or 1. In such instances, when a is 0 the moiety is B- and when a is 1 the moiety is A-B-. Also, a number of moieties disclosed herein exist in multiple tautomeric forms, all of which are intended to be encompassed by any given tautomeric structure.

[0045] The term "hydrocarbyl" refers to a straight, branched, or cyclic alkyl, alkenyl, or alkynyl, each as defined herein. A "C_0" hydrocarbyl is used to refer to a covalent bond. Thus, "C_0-C_3-hydrocarbyl" includes a covalent bond, methyl, ethyl, ethenyl, ethynyl, propyl, propenyl, propynyl, and cyclopropyl.

[0046] The term "alkyl" as employed herein refers to straight and branched chain aliphatic groups having from 1 to 12 carbon atoms, preferably 1-8 carbon atoms, and more preferably 1-6 carbon atoms, which is optionally substituted with one, two or three substituents. Preferred alkyl groups include, without limitation, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, and hexyl. A "C_0" alkyl (as in "C_0-C_3-alkyl") is a covalent bond (like "C_0" hydrocarbyl).

[0047] The term "alkenyl" as used herein means an unsaturated straight or branched chain aliphatic group with one or more carbon-carbon double bonds, having from 2 to 12 carbon atoms, preferably 2-8 carbon atoms, and more preferably 2-6 carbon atoms, which is optionally substituted with one, two or three substituents. Preferred alkenyl groups include, without limitation, ethenyl, propenyl, butenyl, pentenyl, and hexenyl.

[0048] The term "alkynyl" as used herein means an unsaturated straight or branched chain aliphatic group with one or more carbon-carbon triple bonds, having from 2 to 12 carbon atoms, preferably 2-8 carbon atoms, and more preferably 2-6 carbon atoms, which is optionally substituted with one, two or three substituents. Preferred alkynyl groups include, without limitation, ethynyl, propynyl, butynyl, pentynyl, and hexynyl.

[0049] An "alkylene," "alkenylene," or "alkynylene" group is an alkyl, alkenyl, or alkynyl group, as defined hereinabove, that is positioned between and serves to connect two other chemical groups. Preferred alkylene groups include, without limitation, methylene, ethylene, propylene, and butylene. Preferred alkenylene groups include, without limitation, ethenylene, propenylene, and butenylene. Preferred alkynylene groups include, without limitation, ethynylene, propynylene, and butynylene.

[0050] The term "cycloalkyl" as employed herein includes saturated and partially unsaturated cyclic hydrocarbon groups having 3 to 12 carbons, preferably 3 to 8 carbons, and more

preferably 3 to 6 carbons, wherein the cycloalkyl group additionally is optionally substituted. Preferred cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl.

[0051] The term "heteroalkyl" refers to an alkyl group, as defined hereinabove, wherein one or more carbon atoms in the chain are replaced by a heteroatom selected from the group consisting of O, S, and N.

[0052] An "aryl" group is a C_6-C_{14} aromatic moiety comprising one to three aromatic rings, which is optionally substituted. Preferably, the aryl group is a C_6-C_{10} aryl group. Preferred aryl groups include, without limitation, phenyl, naphthyl, anthracenyl, and fluorenyl. An "aralkyl" or "arylalkyl" group comprises an aryl group covalently linked to an alkyl group, either of which may independently be optionally substituted or unsubstituted. Preferably, the aralkyl group is (C_1-C_6)alk(C_6-C_{10})aryl, including, without limitation, benzyl, phenethyl, and naphthylmethyl.

[0053] A "heterocyclyl" or "heterocyclic" group is a ring structure having from about 3 to about 12 atoms, wherein one or more atoms are selected from the group consisting of N, O, and S. The heterocyclic group is optionally substituted on carbon at one or more positions. The heterocyclic group is also independently optionally substituted on nitrogen with alkyl, aryl, aralkyl, alkylcarbonyl, alkylsulfonyl, arylcarbonyl, arylsulfonyl, alkoxycarbonyl, aralkoxycarbonyl, or on sulfur with oxo or lower alkyl. Preferred heterocyclic groups include, without limitation, epoxy, aziridinyl, tetrahydrofuranyl, pyrrolidinyl, piperidinyl, piperazinyl, thiazolidinyl, oxazolidinyl, oxazolidinonyl, and morpholino. In certain preferred embodiments, the heterocyclic group is fused to an aryl, heteroaryl, or cycloalkyl group. Examples of such fused heterocyles include, without limitation, tetrahydroquinoline and dihydrobenzofuran. Specifically excluded from the scope of this term are compounds having an annular O and/or S atom adjacent to another annular O or S.

[0054] As used herein, the term "heteroaryl" refers to groups having 5 to 14 ring atoms, preferably 5, 6, 9, or 10 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to three heteroatoms per ring selected from the group consisting of N, O, and S. The term "heteroaryl" is also meant to encompass monocyclic and bicyclic groups. For example, a heteroaryl group may be pyrimidinyl, pyridinyl, benzimidazolyl, thienyl, benzothiazolyl, benzofuranyl and indolinyl. A "heteroaralkyl" or "heteroarylalkyl" group comprises a heteroaryl group covalently linked to an alkyl group, either of which is independently optionally substituted or unsubstituted. Preferred heteroalkyl groups comprise a C_1-C_6 alkyl group and a heteroaryl group having 5, 6, 9, or 10 ring atoms. Specifically excluded from the scope of this term are compounds having adjacent annular O and/or S atoms.

Examples of preferred heteroaralkyl groups include pyridylmethyl, pyridylethyl, pyrrolylmethyl, pyrrolylethyl, imidazolylmethyl, imidazolylethyl, thiazolylmethyl, and thiazolylethyl. Specifically excluded from the scope of this term are compounds having adjacent annular O and/or S atoms.

[0055] An "arylene," "heteroarylene," or "heterocyclylene" group is an aryl, heteroaryl, or heterocyclyl group, as defined hereinabove, that is positioned between and serves to connect two other chemical groups.

[0056] Preferred heterocyclyls and heteroaryls include, but are not limited to, acridinyl, azocinyl, benzimidazolyl, benzofuranyl, benzothiofuranyl, benzothiophenyl, benzoxazolyl, benzthiazolyl, benztriazolyl, benztetrazolyl, benzisoxazolyl, benzisothiazolyl, benzimidazolinyl, carbazolyl, 4aH-carbazolyl, carbolinyl, chromanyl, chromenyl, cinnolinyl, decahydroquinolinyl, 2H,6H-1,5,2-dithiazinyl, dihydrofuro[2,3-b]tetrahydrofuran, furanyl, furazanyl, imidazolidinyl, imidazolinyl, imidazolyl, 1H-indazolyl, indolenyl, indolinyl, indolizinyl, indolyl, 3H-indolyl, isobenzofuranyl, isochromanyl, isoindazolyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolyl, isoxazolyl, methylenedioxyphenyl, morpholinyl, naphthyridinyl, octahydroisoquinolinyl, oxadiazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, oxazolidinyl, oxazolyl, oxazolidinyl, pyrimidinyl, phenanthridinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, phenoxathiinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, piperidonyl, 4-piperidonyl, piperonyl, pteridinyl, purinyl, pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridooxazole, pyridoimidazole, pyridothiazole, pyridinyl, pyridyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, 2H-pyrrolyl, pyrrolyl, quinazolinyl, quinolinyl, 4H-quinolizinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, tetrazolyl, 6H-1,2,5-thiadiazinyl, 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,2,5-thiadiazolyl, 1,3,4-thiadiazolyl, thianthrenyl, thiazolyl, thienyl, thienothiazolyl, thienooxazolyl, thienoimidazolyl, thiophenyl, triazinyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,5-triazolyl, 1,3,4-triazolyl, and xanthenyl.

[0057] As employed herein, when a moiety (*e.g.*, cycloalkyl, hydrocarbyl, aryl, heteroaryl, heterocyclic, urea, etc.) is described as "optionally substituted" it is meant that the group optionally has from one to four, preferably from one to three, more preferably one or two, non-hydrogen substituents. Suitable substituents include, without limitation, halo, hydroxy, oxo (*e.g.*, an annular -CH- substituted with oxo is -C(O)-) nitro, halohydrocarbyl, hydrocarbyl, aryl, aralkyl, alkoxy, aryloxy, amino, acylamino, alkylcarbamoyl, arylcarbamoyl, aminoalkyl, acyl, carboxy, hydroxyalkyl, , alkanesulfonyl, arenesulfonyl, alkanesulfonamido, arenesulfonamido, aralkylsulfonamido, alkylcarbonyl, acyloxy, cyano, and ureido groups. Preferred substituents, which are themselves not further substituted (unless expressly stated otherwise) are:

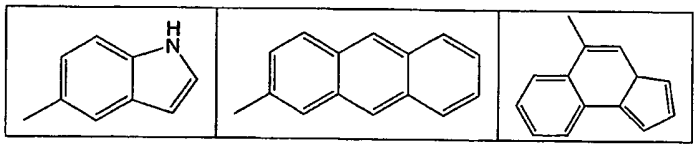
(a) halo, cyano, oxo, carboxy, formyl, nitro, amino, amidino, guanidino,

(b) C_1-C_5 alkyl or alkenyl or arylalkyl imino, carbamoyl, azido, carboxamido, mercapto, hydroxy, hydroxyalkyl, alkylaryl, arylalkyl, C_1-C_8 alkyl, C_1-C_8 alkenyl, C_1-C_8 alkoxy, C_1-C_8 alkoxycarbonyl, aryloxycarbonyl, C_2-C_8 acyl, C_2-C_8 acylamino, C_1-C_8 alkylthio, arylalkylthio, arylthio, C_1-C_8 alkylsulfinyl, arylalkylsulfinyl, arylsulfinyl, C_1-C_8 alkylsulfonyl, arylalkylsulfonyl, arylsulfonyl, C_0-C_6 *N*-alkyl carbamoyl, C_2-C_{15} *N,N*-dialkylcarbamoyl, C_3-C_7 cycloalkyl, aroyl, aryloxy, arylalkyl ether, aryl, aryl fused to a cycloalkyl or heterocycle or another aryl ring, C_3-C_7 heterocycle, or any of these rings fused or spiro-fused to a cycloalkyl, heterocyclyl, or aryl, wherein each of the foregoing is further optionally substituted with one more moieties listed in (a), above; and

(c) $-(CH_2)_s-NR^{30}R^{31}$, wherein s is from 0 (in which case the nitrogen is directly bonded to the moiety that is substituted) to 6, and R^{30} and R^{31} are each independently hydrogen, cyano, oxo, carboxamido, amidino, C_1-C_8 hydroxyalkyl, C_1-C_3 alkylaryl, aryl-C_1-C_3 alkyl, C_1-C_8 alkyl, C_1-C_8 alkenyl, C_1-C_8 alkoxy, C_1-C_8 alkoxycarbonyl, aryloxycarbonyl, aryl-C_1-C_3 alkoxycarbonyl, C_2-C_8 acyl, C_1-C_8 alkylsulfonyl, arylalkylsulfonyl, arylsulfonyl, aroyl, aryl, cycloalkyl, heterocyclyl, or heteroaryl, wherein each of the foregoing is further optionally substituted with one more moieties listed in (a), above; or

R^{30} and R^{31} taken together with the N to which they are attached form a heterocyclyl or heteroaryl, each of which is optionally substituted with from 1 to 3 substituents from (a), above.

[0058] In addition, substituents on cyclic moieties (*i.e.*, cycloalkyl, heterocyclyl, aryl, heteroaryl) include 5-6 membered mono- and 9-14 membered bi-cyclic moieties fused to the parent cyclic moiety to form a bi- or tri-cyclic fused ring system. For example, an optionally substituted phenyl includes the following:



[0059] A "halohydrocarbyl" is a hydrocarbyl moiety in which from one to all hydrogens have been replaced with one or more halo.

[0060] The term "halogen" or "halo" as employed herein refers to chlorine, bromine, fluorine, or iodine. As herein employed, the term "acyl" refers to an alkylcarbonyl or arylcarbonyl

substituent. The term "acylamino" refers to an amide group attached at the nitrogen atom (*i.e.*, R-CO-NH-). The term "carbamoyl" refers to an amide group attached at the carbonyl carbon atom (*i.e.*, NH_2-CO-). The nitrogen atom of an acylamino or carbamoyl substituent is additionally substituted. The term "sulfonamido" refers to a sulfonamide substituent attached by either the sulfur or the nitrogen atom. The term "amino" is meant to include NH_2, alkylamino, arylamino, and cyclic amino groups. The term "ureido" as employed herein refers to a substituted or unsubstituted urea moiety.

[0061] The term "radical" as used herein means a chemical moiety comprising one or more unpaired electrons.

[0062] A moiety that is substituted is one in which one or more hydrogens have been independently replaced with another chemical substituent. As a non-limiting example, substituted phenyls include 2-fluorophenyl, 3,4-dichlorophenyl, 3-chloro-4-fluoro-phenyl, 2-fluor-3-propylphenyl. As another non-limiting example, substituted n-octyls include 2,4 dimethyl-5-ethyl-octyl and 3-cyclopentyl-octyl. Included within this definition are methylenes (-CH_2-) substituted with oxygen to form carbonyl -CO-).

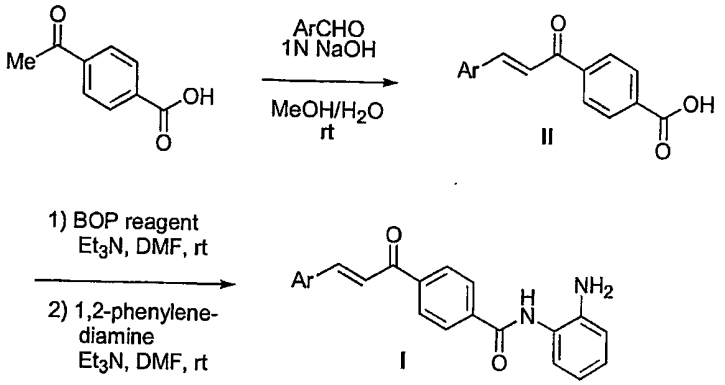
[0063] An "unsubstituted" moiety as defined above (*e.g.*, unsubstituted cycloalkyl, unsubstituted heteroaryl, etc.) means that moiety as defined above that does not have any of the optional substituents for which the definition of the moiety (above) otherwise provides. Thus, for example, while an "aryl" includes phenyl and phenyl substituted with a halo, "unsubstituted aryl" does not include phenyl substituted with a halo.

[0064] Preferred embodiments of the invention also include combinations of the preferred embodiments expressly described herein.

Synthesis

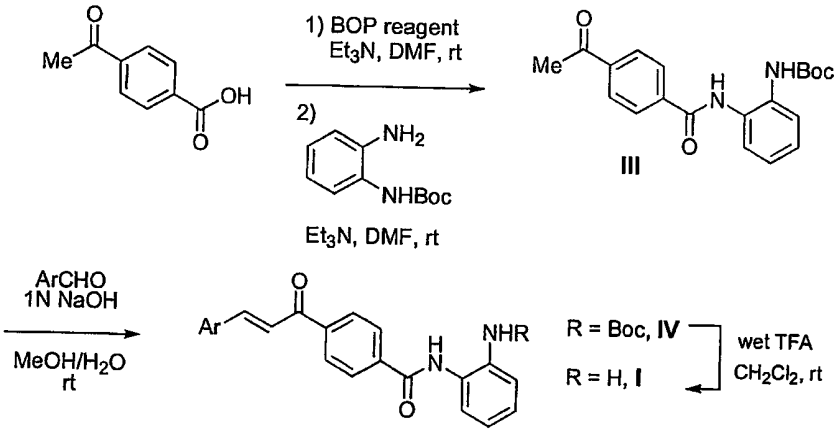
[0065] Compounds of general formula **I** were prepared according to the synthetic routes depicted in Schemes 1 and 2. In some embodiments, 4-acetylbenzoic acid was reacted with an aromatic and/or heteroaromatic aldehyde in a solvent such as methanol (MeOH) in the presence of an aqueous solution of sodium hydroxide (1N) to give after filtration or acidification until pH = 5-6 and filtration, the chalcone **II**. Compound **II** was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N*,*N*-dimethylformamide (DMF) in the presence of triethylamine (Et_3N). The resulting activated ester intermediate formed in situ was finally reacted with 1,2-phenylenediamine to afford the compound **I** (Scheme 1).

Scheme 1



[0066] Alternatively, in some other embodiments, 4-acetylbenzoic acid was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate in a solvent such as *N*,*N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was then reacted with *t*-butyl (2-amino-phenyl)-carbamate to afford the common acetophenone derivative **III**. Chalcone **IV** was prepared by the Claisen-Schmidt condensation of compound **III** with an appropriate aromatic and/or heteroaromatic aldehyde in a solvent such as methanol in the presence of an aqueous solution of sodium hydroxide (1N). *N*-Boc protective group of aniline **IV** was finally cleaved by a wet solution of trifluoroacetic acid (TFA 95% in water) in a solvent such as dichloromethane (CH_2Cl_2) to furnish the compound **I** (Scheme 2).

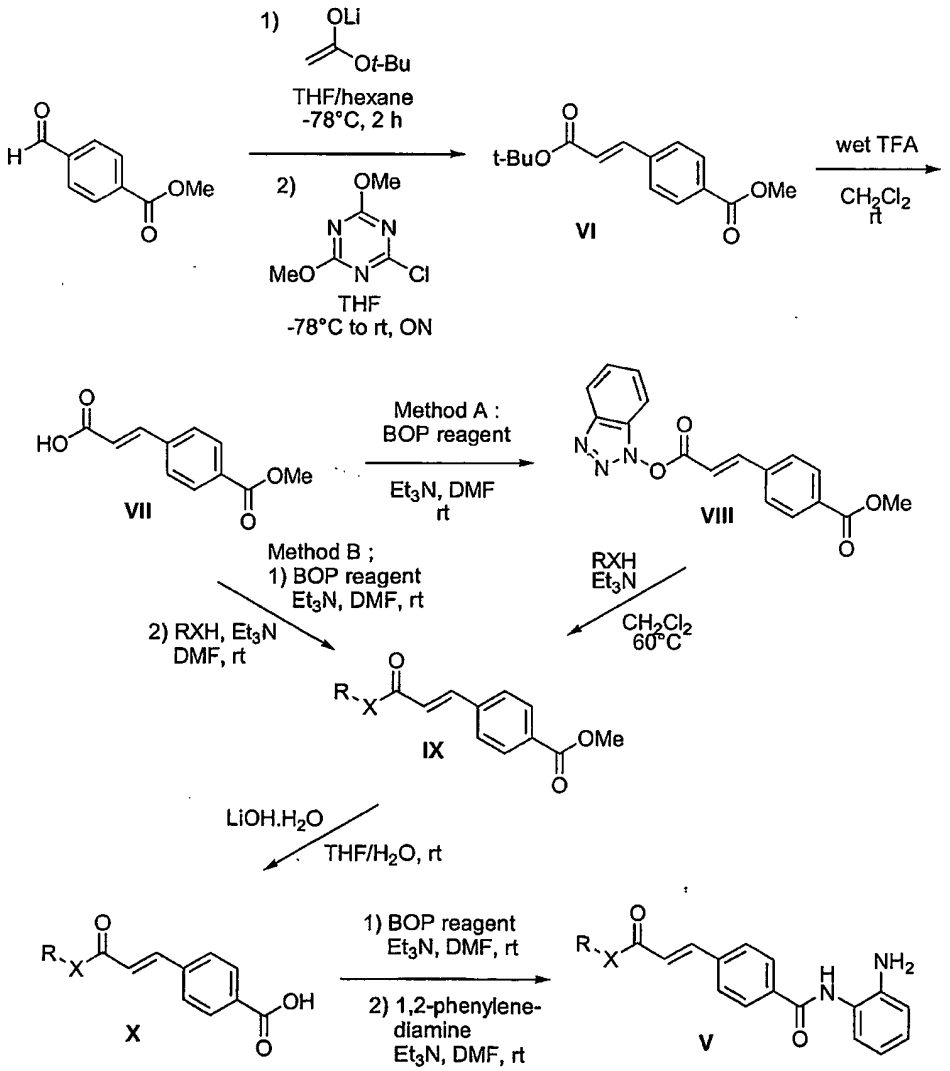
Scheme 2



[0067] Compounds of general formula **V** were prepared according to the synthetic routes depicted in Scheme 3 and 4. In certain preferred embodiments, methyl 4-formylbenzoate was converted into the pure trans-α,β-unsaturated ester **VI** by reaction with the anion of *t*-butyl acetate in a mixture of solvent such as tetrahydrofuran (THF) and hexane followed by the treatment with 2-chloro-4,6-dimethoxy-1,3,5-triazine as a new dehydrating agent. Acidic hydrolysis of *t*-butyl ester **VI** was performed by a wet solution of trifluoroacetic acid (95% in water) in a solvent such as

dichloromethane to give the compound **VII**. The formation of compounds **IX** was carried out by two complementary methods depending of the nucleophilicity of RXH. In the method A, the carboxylic acid **VII** was first converted into the stable activated ester **VIII** by using the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N,N*-dimethylformamide in the presence of triethylamine. This stable activated ester **VIII** was then reacted with a weak nucleophile (*e.g.*, RXH = anilines or aminoheteroaryls) in a solvent such as dichloromethane in the presence of triethylamine to afford the compound **IX**. In the method B, the same activated ester **VIII** intermediate formed in situ from the carboxylic acid **VII**, was then reacted with a strong nucleophile (*e.g.*, RXH = amines, alcohols, thiols, hydroxylamine and derivatives, or hydrazine and derivatives) in a solvent such as *N,N*-dimethylformamide in the presence of triethylamine to afford the compound **IX**.

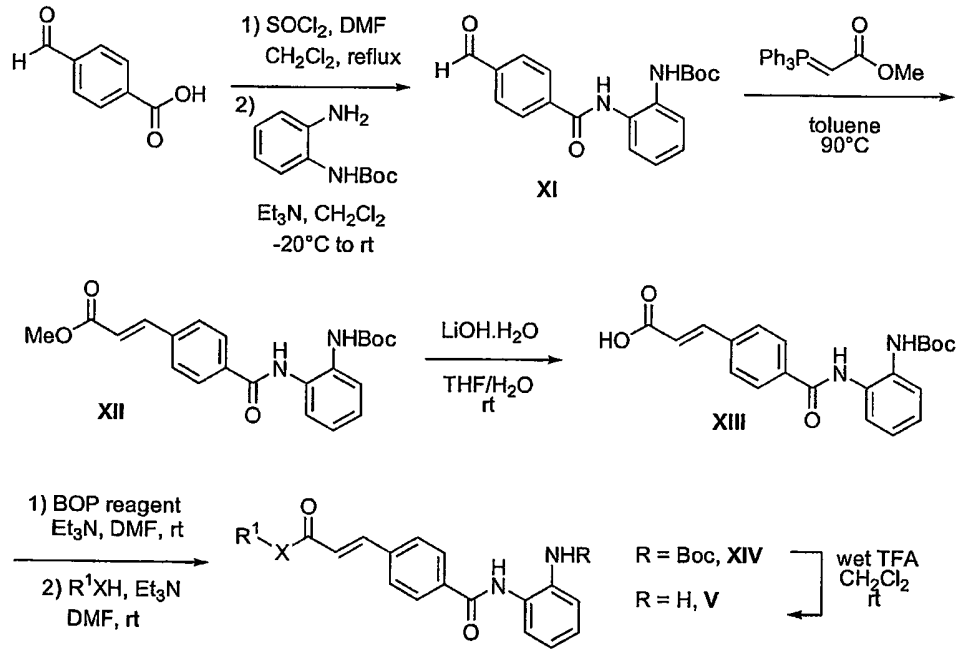
Scheme 3



[0068] Basic hydrolysis of methyl ester **IX** was performed by a aqueous solution of lithium hydroxide in a solvent such as tetrahydrofuran to lead to the compound **X**. Carboxylic acid **X** was finally treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N*,*N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was then reacted with 1,2-phenylenediamine to afford the compound **V** (Scheme 3).

[0069] Alternatively, in some other embodiments, 4-carboxybenzaldehyde was first converted into the acid chloride intermediate by using thionyl chloride ($SOCl_2$) in a solvent such as dichloromethane in the presence of a catalytic amount of *N*,*N*-dimethylformamide. The resulting acid chloride intermediate was then reacted with *t*-butyl (2-amino-phenyl)-carbamate to afford the common benzaldehyde derivative **XI**.

Scheme 4

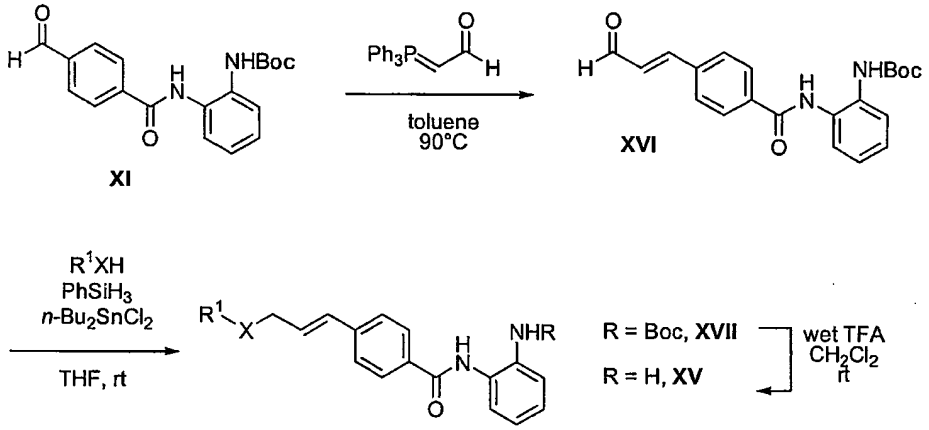


[0070] Wittig olifination of the aldehyde **XI** was performed with methyl (triphenyl-phosphoranylidene)acetate in a solvent such as toluene to provide the *trans*-α,β-unsaturated ester **XII**. Basic hydrolysis of methyl ester **XII** was performed by a aqueous solution of lithium hydroxide in a solvent such as tetrahydrofuran to lead to the compound **XIII**. Carboxylic acid **XIII** was treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N*,*N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was then reacted with the nucleophile R^1XH to afford the compound **XIV**. *N*-Boc protective group of aniline **XIV** was

finally cleaved by a wet solution of trifluoroacetic acid (95% in water) in a solvent such as dichloromethane to furnish the compound **V** (Scheme 4).

[0071] Compounds of general formula **XV** were prepared according to the synthetic routes depicted in Schemes 5-7. Thus, Wittig olifination of aldehyde **XI** was performed with the (triphenylphosphoranylidene)-acetaldehyde reagent in a solvent such as toluene to provide the *trans*-α,β-unsaturated aldehyde **XVI**. In certain preferred embodiments, the formation of compounds **XVII** was performed by a reductive amination following the described method depending of the nucleophilicity of R^1XH. Aldehyde **XVI** was first mixed with a weak nucleophile (*e.g.*, R^1XH = anilines or aminoheteroaryls) in a solvent such as tetrahydrofuran in the presence of a catalytic amount of dibutyltin dichloride. The resulting iminium intermediate formed in situ was then reacted with the reductive reagent phenylsilane to afford the compound **XVII**.

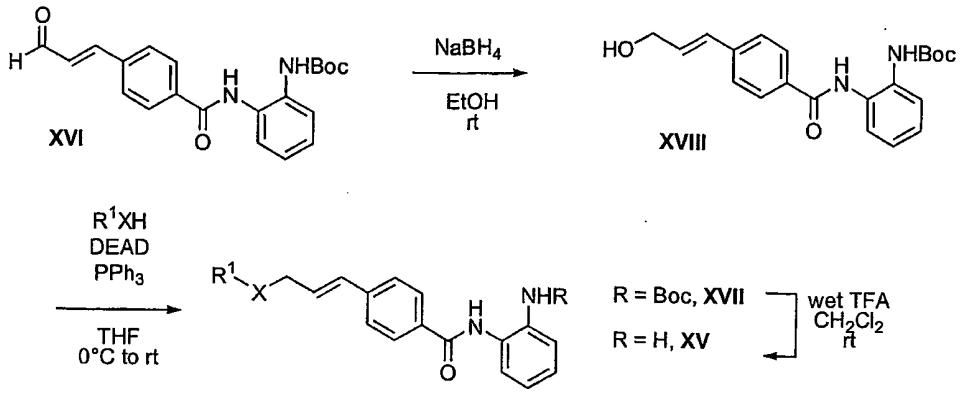
Scheme 5



[0072] *N*-Boc protective group of aniline **XVII** was finally cleaved by a wet solution of trifluoroacetic acid (95% in water) in a solvent such as dichloromethane to furnish the compound **XV** (Scheme 5).

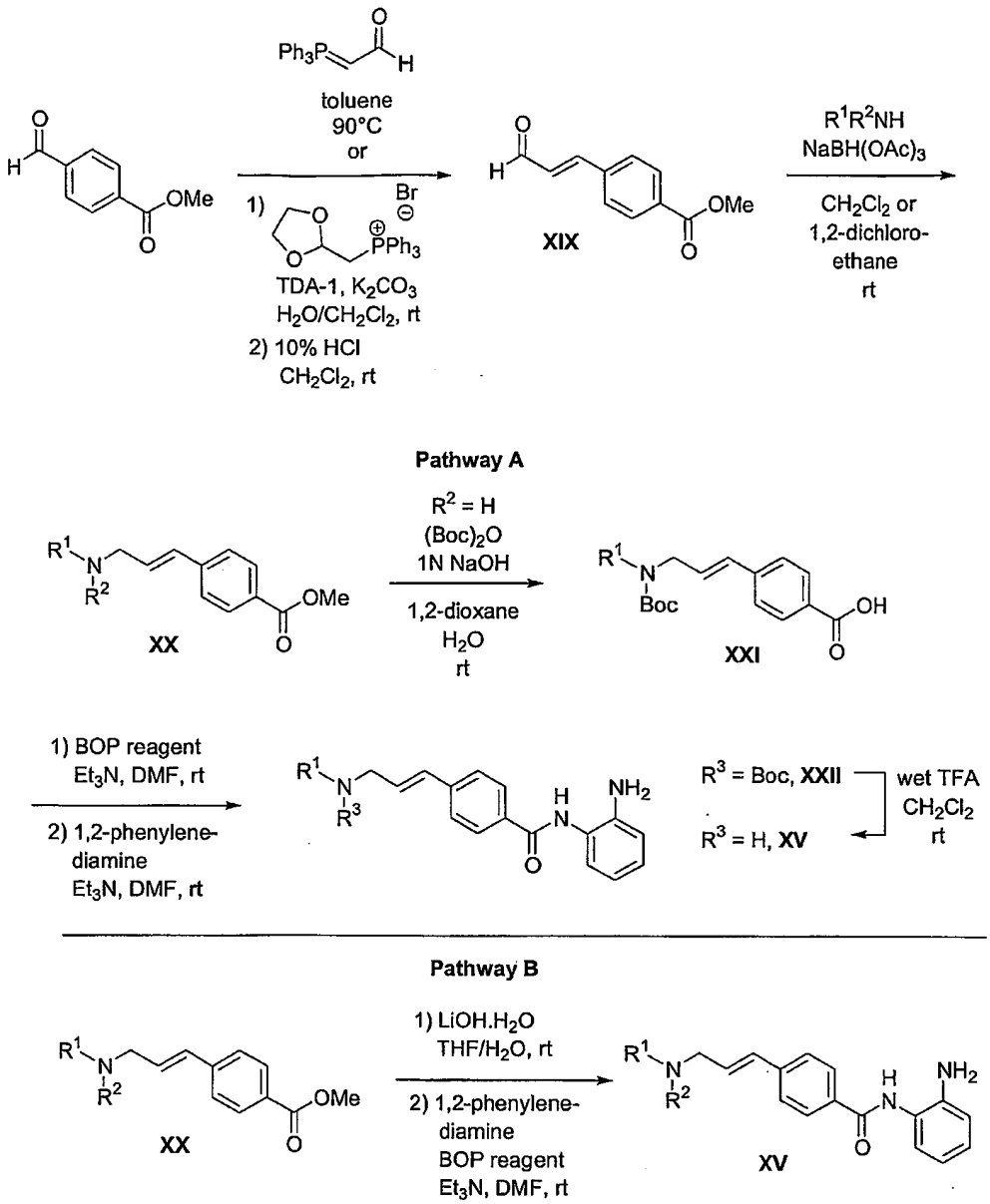
[0073] Alternatively, in some other embodiments, the *trans*-α,β-unsaturated aldehyde **XVI** was reduced into the primary allylic alcohol **XVIII** by the reductive reagent sodium borohydride in a solvent such as ethanol. This alcohol **XVIII** was then reacted with a nucleophile R^1XH according to a Mitsunobu type reaction in a solvent such as tetrahydrofuran in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) to furnish the compound **XVII**. *N*-Boc protective group of aniline **XVII** was finally cleaved by a wet solution of trifluoroacetic acid (95% in water) in a solvent such as dichloromethane to furnish the compound **XV** (Scheme 6).

Scheme 6



[0074] Moreover, in some other embodiments, Wittig olefination of methyl 4-formylbenzoate was performed using either the (triphenylphosphoranylidene)-acetaldehyde reagent in a solvent such as toluene or the (1,3-dioxolan-2-yl)methyltriphenylphosphonium bromide reagent in the presence of TDA-1 {tris[2-(2-methoxyethoxy)ethyl]amine) and potassium carbonate in a biphasic medium such as dichloromethane/water followed by an acidic hydrolysis to provide the *trans*-α,β-unsaturated aldehyde **XIX**. Aldehyde **XIX** was first mixed with a nucleophile (R^1R^2NH) in a solvent such as dichloromethane or 1,2-dichloroethane. The resulting iminium intermediate formed in situ was then reacted with the reductive reagent sodium triacetoxyborohydride [$NaBH(OAc)_3$] to afford the compound **XX**. In the pathway A, the basic hydrolysis of the methyl ester and protection of the secondary amine of compound **XX** (R^1 = alkyl, R^2 = H) were performed at the same time in the presence of an aqueous solution of sodium hydroxide (1N) and the protective reagent di-*tert*-butyl dicarbonate [$(Boc)_2O$] in a solvent such 1,4-dioxane to lead to the compound **XXI**. Carboxylic acid **XXI** was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N*,*N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was then reacted with 1,2-phenylenediamine to afford the compound **XXII**. *N*-Boc protective group of amine **XXII** was finally cleaved by a solution of wet trifluoroacetic acid (95% in water) in a solvent such as dichloromethane to furnish the compound **XV** (Scheme 7).

Scheme 7

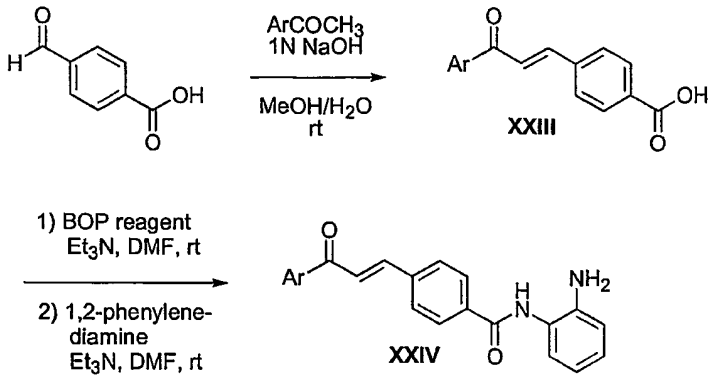


[0075] In the pathway B, the methyl ester **XX** was directly converted into the final compound **XV** after basic hydrolysis and coupling with 1,2-phenylenediamine (Scheme 7).

[0076] Compounds of general formula **XXIV** were prepared according to the synthetic routes depicted in Schemes 8 and 9. In some embodiments, 4-formylbenzoic acid was reacted with an aryl and/or heteroaryl methyl ketone in a solvent such as methanol (MeOH) in the presence of an aqueous solution of sodium hydroxide (1N) to give after filtration the chalcone **XXIII**. Compoud **XXIII** was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N,N*-

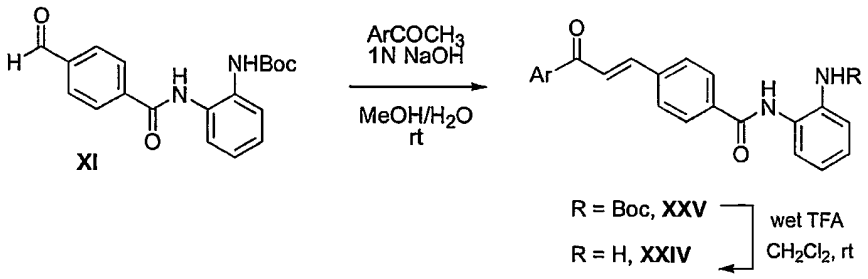
dimethylformamide (DMF) in the presence of triethylamine (Et_3N). The resulting activated ester intermediate formed in situ was finally reacted with 1,2-phenylenediamine to afford the compound **XXIV** (Scheme 8).

Scheme 8



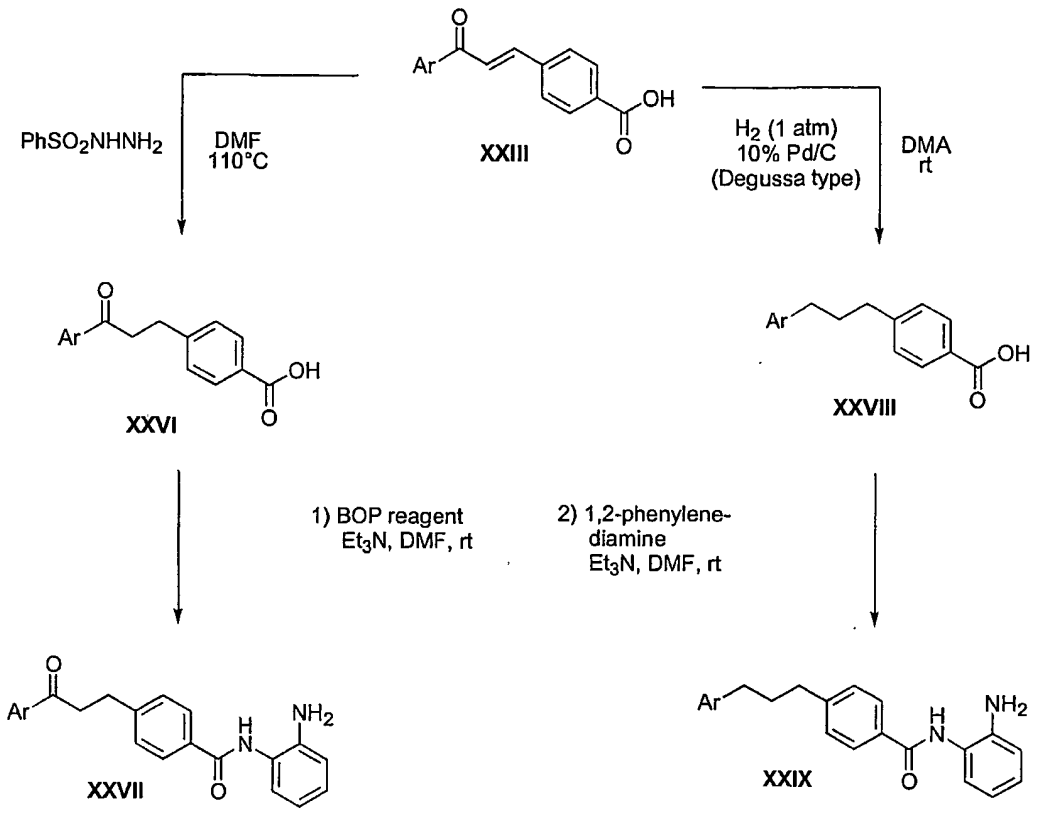
[0077] Alternatively, in some other embodiments, chalcone **XXV** was prepared by the Claisen-Schmidt condensation of benzaldehyde derivative **XI** with an appropriate aryl and/or heteroaryl methyl ketone in a solvent such as methanol in the presence of an aqueous solution of sodium hydroxide (1N). *N*-Boc protective group of aniline **XXV** was finally cleaved by a wet solution of trifluoroacetic acid (TFA 95% in water) in a solvent such as dichloromethane (CH_2Cl_2) to furnish the compound **XXIV** (Scheme 9).

Scheme 9



[0078] Compounds of general formula **XXVII** and **XXIX** were prepared according to the synthetic routes depicted in Scheme 10. Thus, selective reduction of the double bond of compound **XXIII** was carried out by using the reductive reagent benzenesulfonyl hydrazide in a solvent such as *N*,*N*-dimethylformamide to produce compound **XXVI**. Carboxylic acid **XXVI** was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N*,*N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was finally reacted with 1,2-phenylenediamine to afford the compound **XXVII**.

Scheme 10

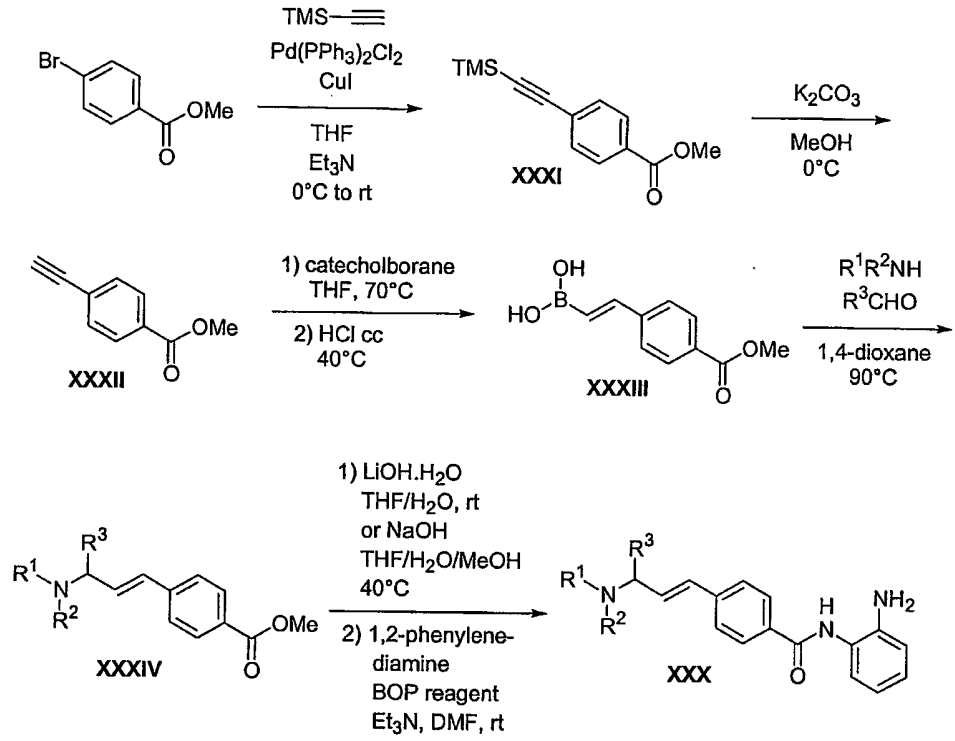


[0079] Moreover, the complete reduction of the α,β-unsaturated ketone **XXIII** into the saturated compound **XXVIII** was performed by an hydrogenation catalyzed by 10% of palladium on charcoal (Degussa type) in a solvent such as *N,N*-dimethylacetamide (DMA). Then, the carboxylic acid **XXVIII** was first treated with the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in a solvent such as *N,N*-dimethylformamide in the presence of triethylamine. The resulting activated ester intermediate formed in situ was finally reacted with 1,2-phenylenediamine to afford the compound **XXIX** (Scheme 10).

[0080] Compounds of general formula **XXX** were prepared according to the synthetic route depicted in Scheme 11. Thus, Sonogashira type reaction between methyl 4-bromobenzoate and (trimethylsilyl)acetylene was carried out by a catalytic amount of palladium catalyst and copper iodide in the presence of Et_3N in a solvent such THF to afford the protected alkyne **XXXI**. Basic deprotection of TMS group of **XXXI** was performed by potassium carbonate in the presence of methanol to give the alkyne **XXXII**. Hydroboration of the triple bond of **XXXII** was performed by the catecholborane reagent in a solvent such THF followed by acidic hydrolysis of the boronate intermediate to furnish boronic acid **XXXIII**. Allylic amine **XXXIV** was elaborated according to the

Petasis type reaction by reacting the vinylboronic acid **XXXIII** with a pre-formed mixture between an amino compound (R^1R^2NH) and an aldehyde (R^3CHO) in a solvent such 1,4-dioxane. Finally, the methyl ester **XXIV** was converted into the final compound **XXX** after basic hydrolysis and coupling with 1,2-phenylenediamine.

Scheme 11



Inhibition of Histone Deacetylase

[0081] In a third aspect, the invention provides a method of inhibiting histone deacetylase in a cell, comprising contacting a cell in which inhibition of histone deacetylase is desired with an inhibitor of histone deacetylase according to the invention.

[0082] Measurement of the enzymatic activity of a histone deacetylase can be achieved using known methodologies. For example, Yoshida et al., J. Biol. Chem., **265**: 17174-17179 (1990), describes the assessment of histone deacetylase enzymatic activity by the detection of acetylated histones in trichostatin A treated cells. Taunton et al., Science, **272**: 408-411 (1996), similarly describes methods to measure histone deacetylase enzymatic activity using endogenous and recombinant HDAC-1.

[0083] In some preferred embodiments, the histone deacetylase inhibitor interacts with and reduces the activity of all histone deacetylases in the cell. In some other preferred embodiments according to this aspect of the invention, the histone deacetylase inhibitor interacts with and

reduces the activity of fewer than all histone deacetylases in the cell. In certain preferred embodiments, the inhibitor interacts with and reduces the activity of one histone deacetylase (*e.g.*, HDAC-1), but does not interact with or reduce the activities of other histone deacetylases (*e.g.*, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC-7, and HDAC-8). As discussed below, certain particularly preferred histone deacetylase inhibitors are those that interact with, and reduce the enzymatic activity of, a histone deacetylase that is involved in tumorigenesis. Certain other preferred histone deacetylase inhibitors interact with and reduce the enzymatic activity of a fungal histone deacetylase.

[0084] Preferably, the method according to the third aspect of the invention causes an inhibition of cell proliferation of the contacted cells. The phrase "inhibiting cell proliferation" is used to denote an ability of an inhibitor of histone deacetylase to retard the growth of cells contacted with the inhibitor as compared to cells not contacted. An assessment of cell proliferation can be made by counting contacted and non-contacted cells using a Coulter Cell Counter (Coulter, Miami, FL) or a hemacytometer. Where the cells are in a solid growth (*e.g.*, a solid tumor or organ), such an assessment of cell proliferation can be made by measuring the growth with calipers and comparing the size of the growth of contacted cells with non-contacted cells.

[0085] Preferably, growth of cells contacted with the inhibitor is retarded by at least 50% as compared to growth of non-contacted cells. More preferably, cell proliferation is inhibited by 100% (*i.e.*, the contacted cells do not increase in number). Most preferably, the phrase "inhibiting cell proliferation" includes a reduction in the number or size of contacted cells, as compared to non-contacted cells. Thus, an inhibitor of histone deacetylase according to the invention that inhibits cell proliferation in a contacted cell may induce the contacted cell to undergo growth retardation, to undergo growth arrest, to undergo programmed cell death (*i.e.*, to apoptose), or to undergo necrotic cell death.

[0086] The cell proliferation inhibiting ability of the histone deacetylase inhibitors according to the invention makes them useful research tools to study the role of histone deacetylase in various biological processes. For example, the cell proliferation inhibiting ability of the histone deacetylase inhibitors according to the invention allow the synchronization of a population of asynchronously growing cells. For example, the histone deacetylase inhibitors of the invention may be used to arrest a population of non-neoplastic cells grown *in vitro* in the G1 or G2 phase of the cell cycle. Such synchronization allows, for example, the identification of gene and/or gene products expressed during the G1 or G2 phase of the cell cycle. Such synchronization of cultured

cells may also be useful for testing the efficacy of a new transfection protocol, where transfection efficiency varies and is dependent upon the particular cell cycle phase of the cell to be transfected. Use of the histone deacetylase inhibitors of the invention allows the synchronization of a population of cells, thereby aiding detection of enhanced transfection efficiency.

[0087] In some preferred embodiments, the contacted cell is a neoplastic cell. The term "neoplastic cell" is used to denote a cell that shows aberrant cell growth. Preferably, the aberrant cell growth of a neoplastic cell is increased cell growth. A neoplastic cell may be a hyperplastic cell, a cell that shows a lack of contact inhibition of growth *in vitro*, a benign tumor cell that is incapable of metastasis in vivo, or a cancer cell that is capable of metastasis in vivo and that may recur after attempted removal. The term "tumorigenesis" is used to denote the induction of cell proliferation that leads to the development of a neoplastic growth. In some embodiments, the histone deacetylase inhibitor induces cell differentiation in the contacted cell. Thus, a neoplastic cell, when contacted with an inhibitor of histone deacetylase may be induced to differentiate, resulting in the production of a non-neoplastic daughter cell that is phylogenetically more advanced than the contacted cell.

[0088] In some preferred embodiments, the contacted cell is in an animal. Thus, the invention provides a method for treating a cell proliferative disease or condition in an animal, comprising administering to an animal in need of such treatment a therapeutically effective amount of a histone deacetylase inhibitor of the invention. Preferably, the animal is a mammal, more preferably a domesticated mammal. Most preferably, the animal is a human.

[0089] The term "cell proliferative disease or condition" is meant to refer to any condition characterized by aberrant cell growth, preferably abnormally increased cellular proliferation. Examples of such cell proliferative diseases or conditions include, but are not limited to, cancer, restenosis, and psoriasis. In particularly preferred embodiments, the invention provides a method for inhibiting neoplastic cell proliferation in an animal comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a histone deacetylase inhibitor of the invention.

[0090] It is contemplated that some compounds of the invention have inhibitory activity against a histone deacetylase from a protozoal source. Thus, the invention also provides a method for treating or preventing a protozoal disease or infection, comprising administering to an animal in need of such treatment a therapeutically effective amount of a histone deacetylase inhibitor of the invention. Preferably the animal is a mammal, more preferably a human. Preferably, the histone deacetylase inhibitor used according to this embodiment of the invention

inhibits a protozoal histone deacetylase to a greater extent than it inhibits mammalian histone deacetylases, particularly human histone deacetylases.

[0091] The present invention further provides a method for treating a fungal disease or infection comprising administering to an animal in need of such treatment a therapeutically effective amount of a histone deacetylase inhibitor of the invention. Preferably the animal is a mammal, more preferably a human. Preferably, the histone deacetylase inhibitor used according to this embodiment of the invention inhibits a fungal histone deacetylase to a greater extent than it inhibits mammalian histone deacetylases, particularly human histone deacetylases.

[0092] The term "therapeutically effective amount" is meant to denote a dosage sufficient to cause inhibition of histone deacetylase activity in the cells of the subject, or a dosage sufficient to inhibit cell proliferation or to induce cell differentiation in the subject. Administration may be by any route, including, without limitation, parenteral, oral, sublingual, transdermal, topical, intranasal, intratracheal, or intrarectal. In certain particularly preferred embodiments, compounds of the invention are administered intravenously in a hospital setting. In certain other preferred embodiments, administration may preferably be by the oral route.

[0093] When administered systemically, the histone deacetylase inhibitor is preferably administered at a sufficient dosage to attain a blood level of the inhibitor from about 0.01 μM to about 100 μM, more preferably from about 0.05 μM to about 50 μM, still more preferably from about 0.1 μM to about 25 μM, and still yet more preferably from about 0.5 μM to about 25 μM. For localized administration, much lower concentrations than this may be effective, and much higher concentrations may be tolerated. One of skill in the art will appreciate that the dosage of histone deacetylase inhibitor necessary to produce a therapeutic effect may vary considerably depending on the tissue, organ, or the particular animal or patient to be treated.

[0094] In certain preferred embodiments of the third aspect of the invention, the method further comprises contacting the cell with an antisense oligonucleotide that inhibits the expression of a histone deacetylase. The combined use of a nucleic acid level inhibitor (*e.g.*, antisense oligonucleotide) and a protein level inhibitor (*i.e.*, inhibitor of histone deacetylase enzyme activity) results in an improved inhibitory effect, thereby reducing the amounts of the inhibitors required to obtain a given inhibitory effect as compared to the amounts necessary when either is used individually. The antisense oligonucleotides according to this aspect of the invention are complementary to regions of RNA or double-stranded DNA that encode HDAC-1, HDAC-2, HDAC-3, HDAC-4, HDAC-5, HDAC-6, HDAC7, and/or HDAC-8 (see *e.g.*, GenBank Accession Number

U50079 for HDAC-1, GenBank Accession Number U31814 for HDAC-2, and GenBank Accession Number U75697 for HDAC-3).

[0095] For purposes of the invention, the term "oligonucleotide" includes polymers of two or more deoxyribonucleosides, ribonucleosides, or 2'-substituted ribonucleoside residues, or any combination thereof. Preferably, such oligonucleotides have from about 6 to about 100 nucleoside residues, more preferably from about 8 to about 50 nucleoside residues, and most preferably from about 12 to about 30 nucleoside residues. The nucleoside residues may be coupled to each other by any of the numerous known internucleoside linkages. Such internucleoside linkages include without limitation phosphorothioate, phosphorodithioate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate and sulfone internucleoside linkages. In certain preferred embodiments, these internucleoside linkages may be phosphodiester, phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof. The term oligonucleotide also encompasses such polymers having chemically modified bases or sugars and/ or having additional substituents, including without limitation lipophilic groups, intercalating agents, diamines and adamantane.

[0096] For purposes of the invention the term "2'-substituted ribonucleoside" includes ribonucleosides in which the hydroxyl group at the 2' position of the pentose moiety is substituted to produce a 2'-O-substituted ribonucleoside. Preferably, such substitution is with a lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl or allyl group may be unsubstituted or may be substituted, *e.g.*, with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups. The term "2'-substituted ribonucleoside" also includes ribonucleosides in which the 2'-hydroxyl group is replaced with an amino group or with a halo group, preferably fluoro.

[0097] Particularly preferred antisense oligonucleotides utilized in this aspect of the invention include chimeric oligonucleotides and hybrid oligonucleotides.

[0098] For purposes of the invention, a "chimeric oligonucleotide" refers to an oligonucleotide having more than one type of internucleoside linkage. One preferred example of such a chimeric oligonucleotide is a chimeric oligonucleotide comprising a phosphorothioate, phosphodiester or phosphorodithioate region, preferably comprising from about 2 to about 12 nucleotides, and an alkylphosphonate or alkylphosphonothioate region (see *e.g.*, Pederson et al. U.S. Patent Nos.

5,635,377 and 5,366,878). Preferably, such chimeric oligonucleotides contain at least three consecutive internucleoside linkages selected from phosphodiester and phosphorothioate linkages, or combinations thereof.

[0099] For purposes of the invention, a "hybrid oligonucleotide" refers to an oligonucleotide having more than one type of nucleoside. One preferred example of such a hybrid oligonucleotide comprises a ribonucleotide or 2'-substituted ribonucleotide region, preferably comprising from about 2 to about 12 2'-substituted nucleotides, and a deoxyribonucleotide region. Preferably, such a hybrid oligonucleotide contains at least three consecutive deoxyribonucleosides and also contains ribonucleosides, 2'-substituted ribonucleosides, preferably 2'-O-substituted ribonucleosides, or combinations thereof (see *e.g.*, Metelev and Agrawal, U.S. Patent No. 5,652,355).

[0100] The exact nucleotide sequence and chemical structure of an antisense oligonucleotide utilized in the invention can be varied, so long as the oligonucleotide retains its ability to inhibit expression of the gene of interest. This is readily determined by testing whether the particular antisense oligonucleotide is active. Useful assays for this purpose include quantitating the mRNA encoding a product of the gene, a Western blotting analysis assay for the product of the gene, an activity assay for an enzymatically active gene product, or a soft agar growth assay, or a reporter gene construct assay, or an in vivo tumor growth assay, all of which are described in detail in this specification or in Ramchandani et al. (1997) Proc. Natl. Acad. Sci. USA 94: 684-689.

[0101] Antisense oligonucleotides utilized in the invention may conveniently be synthesized on a suitable solid support using well known chemical approaches, including H-phosphonate chemistry, phosphoramidite chemistry, or a combination of H-phosphonate chemistry and phosphoramidite chemistry (*i.e.*, H-phosphonate chemistry for some cycles and phosphoramidite chemistry for other cycles). Suitable solid supports include any of the standard solid supports used for solid phase oligonucleotide synthesis, such as controlled-pore glass (CPG) (see, *e.g.*, Pon, R.T. (1993) Methods in Molec. Biol. 20: 465-496).

[0102] Particularly preferred oligonucleotides have nucleotide sequences of from about 13 to about 35 nucleotides which include the nucleotide sequences shown in Table 1. Yet additional particularly preferred oligonucleotides have nucleotide sequences of from about 15 to about 26 nucleotides of the nucleotide sequences shown in Table 1.

Table 1

Oligo	Target	Accession Number	Nucleotide Position	Sequence	position within Gene
HDAC1 AS1	Human HDAC1	U50079	1585-1604	5'-GAAACGTGAGGGACTCAGCA-3'	3'-UTR
HDAC1 AS2	Human HDAC1	U50079	1565-1584	5'-GGAAGCCAGAGCTGGAGAGG-3'	3'-UTR
HDAC1 MM	Human HDAC1	U50079	1585-1604	5'-GTTAGGTGAGGCACTGAGGA-3'	3'-UTR
HDAC2 AS	Human HDAC2	U31814	1643-1622	5'-GCTGAGCTGTTCTGATTTGG-3'	3'-UTR
HDAC2 MM	Human HDAC2	U31814	1643-1622	5'-CGTGAGCACTTCTCATTTCC-3'	3'-UTR
HDAC3 AS	Human HDAC3	AF039703	1276-1295	5'-CGCTTTCCTTGTCATTGACA-3'	3'-UTR
HDAC3 MM	Human HDAC3	AF039703	1276-1295	5'-GCCTTTCCTACTCATTGTGT-3'	3'-UTR
HDAC4 AS1	Human HDAC4	AB006626	514-33	5-GCTGCCTGCCGTGCCCACCC-3'	5'-UTR
HDAC4 MM1	Human HDAC4	AB006626	514-33	5'-CGTGCCTGCGCTGCCCACGG-3'	5'-UTR
HDAC4 AS2	Human HDAC4	AB006626	7710-29	5'-TACAGTCCATGCAACCTCCA-3'	3'-UTR
HDAC4 MM4	Human HDAC4	AB006626	7710-29	5'-ATCAGTCCAACCAACCTCGT-3'	3'-UTR
HDAC5 AS	Human HDAC5	AF039691	2663-2682	5'-CTTCGGTCTCACCTGCTTGG-3'	3'-UTR
HDAC6 AS	Human HDAC6	AJ011972	3791-3810	5'-CAGGCTGGAATGAGCTACAG-3'	3'-UTR
HDAC6 MM	Human HDAC6	AJ011972	3791-3810	5'-GACGCTGCAATCAGGTAGAC-3'	3'-UTR
HDAC7 AS	Human HDAC7	AF239243	2896-2915	5'-CTTCAGCCAGGATGCCCACA-3'	3'-UTR
HDAC8 AS1	Human HDAC8	AF230097	51-70	5'-CTCCGGCTCCTCCATCTTCC-3'	5'-UTR
HDAC8 AS2	Human HDAC8	AF230097	1328-1347	5'-AGCCAGCTGCCACTTGATGC-3'	3'-UTR

[0103] The following examples are intended to further illustrate certain preferred embodiments of the invention, and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

N-(2-Amino-phenyl)-4-[3-(3,4-dichloro-phenyl)-acryloyl]-benzamide (Ia)

Step 1: 4-[3-(3,4-dichloro-phenyl)-acryloyl]-benzoic acid (**IIa**)

[0104] To a stirred suspension at room temperature of 4-acetylbenzoic acid (1.71 g, 10.44 mmol), 3,4-dichlorobenzaldehyde (2.05 g, 11.49 mmol) or aldehyde (1.1 equiv.) in MeOH (50 ml) was added a solution of NaOH (26.1 ml, 1N in H_2O). After 19 h, the reaction mixture was filtered off, rinsed with MeOH and dried to afford the title compound **IIa** (3.22 g, 10.03 mmol, 96% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 8.35 (s, 1H), AB system (δ_A = 8.13, δ_B = 8.01, J = 8.4 Hz, 4H), 8.12 (d, J = 15.8 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.73 (d, 15.8 Hz, 1H).

Step 2: *N*-(2-Amino-phenyl)-4-[3-(3,4-dichloro-phenyl)-acryloyl]-benzamide (**Ia**)

[0105] To a stirred solution at room temperature of **IIa** (300 mg, 0.93 mmol) in anhydrous DMF (15 ml) under nitrogen were added Et_3N (156 µl, 1.12 mmol) and BOP reagent (454 mg, 1.03 mmol), respectively. After 30 min, a solution of 1,2-phenylenediamine (111 mg, 1.03 mmol), Et_3N (391 µl, 2.80 mmol) in anhydrous DMF (2 ml) was added dropwise. After 21 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/CH_2Cl_2 : 10/90→20/90) to afford the title compound **Ia** (237 mg, 0.58 mmol, 62% yield) as a yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.90 (s, 1H), 8.40-8.30 (m, 3H), 8.25-8.10 (m, 3H), 7.97 (d, J = 8.8 Hz, 1H), 7.85-7.75 (m, 2H), 7.23 (d, J = 7.5 Hz, 1H), 7.03 (t, J = 7.3 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.65 (t, J = 7.5 Hz, 1H), 4.99 (s, 2H).

Examples 2 and 10

[0106] **Examples 2 and 10** (compounds **Ib,Ij**) were prepared using the same procedure as described for compound **Ia** of **Example 1** (Scheme 1).

Example 3

*N-(2-Amino-phenyl)-4-[3-(2,6-dichloro-phenyl)-acryloyl]-benzamide (**Ic**)*

Step 1: t-Butyl [2-(4-acetyl-benzoylamino)-phenyl]-carbamate (**III**)

[0107] To a stirred solution at room temperature of 4-acetylbenzoic acid (395 mg, 2.41 mmol) in anhydrous DMF (15 ml) under nitrogen were added Et_3N (369 µl, 2.65 mmol) and BOP reagent (1.171 g, 2.65 mmol), respectively. After 30 min, a solution of t-butyl (2-amino-phenyl)-carbamate (551 mg, 2.65 mmol), Et_3N (1.01 ml, 7.22 mmol) in anhydrous DMF (5 ml) was added dropwise. After 19 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane : 40/60→50/50) to afford the title compound **III** (500 mg, 1.41 mmol, 59% yield) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : 9.47 (bs, 1H), 8.10-8.00 (m, 4H), 7.91 (d, J = 7.9 Hz, 1H), 7.33-7.15 (m, 3H), 6.67 (s, 1H), 2.67 (s, 3H), 1.53 (s, 9H).

Step 2: t-Butyl (2-{4-[3-(2,6-dichloro-phenyl)-acryloyl]-benzoylamino}-phenyl)-carbamate (**IVc**)

[0108] To a stirred solution at room temperature of **III** (150 mg, 0.42 mmol), 2,6-dichlorobenzaldehyde (148 mg, 0.85 mmol) or aldehyde (1.5-2.0 equiv.) in MeOH (10 ml) was added a solution of NaOH (1.7 ml, 1N in H_2O). A pale yellow precipitate appeared. After 3 days, the reaction mixture was filtered off, rinsed with H_2O. The solid residue was then dissolved in AcOEt, dried over anhydrous $MgSO_4$, filtered and concentrated. The crude residue was finally purified by flash chromatography on silica gel (AcOEt/hexane : 20/80→40/60) to afford the title compound **IVc** (185 mg, 0.36 mmol, 85% yield) as a pale yellow foam. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : 9.48 (bs, 1H), 8.11 (s, 4H), 7.96 (d, J = 17.1 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 16.3 Hz, 1H), 7.42 (d, J = 7.9 Hz, 2H), 7.35-7.15 (m, 4H), 6.68 (s, 1H), 1.54 (s, 9H).

Step 3: N-(2-Amino-phenyl)-4-[3-(2,6-dichloro-phenyl)-acryloyl]-benzamide (**Ic**)

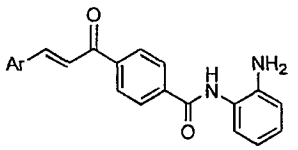
[0109] To a stirred solution at room temperature of **IVc** (135 mg, 0.26 mmol) in CH_2Cl_2 (10 ml) was added trifluoroacetic acid (2 ml, 95% in water). After 16 h, the reaction mixture was concentrated, and directly purified by flash chromatography on silica gel (AcOEt/CH_2Cl_2 : 15/85) to afford the title compound **Ic** (90 mg, 0.22 mmol, 83% yield) as an orange solid. ^1H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.89 (s, 1H), AB system (δ_A = 8.23, δ_B = 8.19, J = 8.5 Hz, 4H), 7.90 (d, J = 16.3

Hz, 1H), 7.78 (d, J = 16.3 Hz, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.55-7.45 (m, 1H), 7.23 (d, J = 7.5 Hz, 1H), 7.03 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 5.00 (s, 2H).

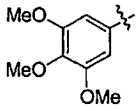
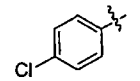
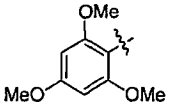
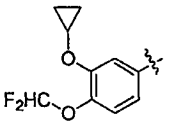
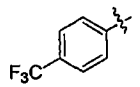
Examples 4-9

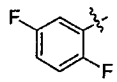
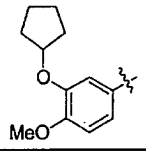
[0110] **Examples 4 to 9** (compounds **Id-Ii**) were prepared using the same procedure as described for compound **Ic** of **Example 3** (Scheme 2).

Table 2



I

Cmpd	Ar	Name	Characterization	Scheme
Ib		*N*-(2-Amino-phenyl)-4-(3-pyridin-3-yl-acryloyl)-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.89 (s, 1H), 9.10 (s, 1H), 8.68 (d, J = 4.8 Hz, 1H), 8.44 (d, J = 7.9 Hz, 1H), AB system (δ_A = 8.34, δ_B = 8.20, J = 8.1 Hz, 4H), 8.19 (d, J = 15.8 Hz, 1H), 7.87 (d, J = 15.8 Hz, 1H), 7.60-7.50 (m, 1H), 7.23 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 7.0 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.65 (t, J = 7.3 Hz, 1H), 4.99 (s, 2H).	1
Id		*N*-(2-Amino-phenyl)-4-[3-(3,4,5-trimethoxy-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.89 (s, 1H), AB system (δ_A = 8.31, δ_B = 8.20, J = 8.4 Hz, 4H), 7.98 (d, J = 15.8 Hz, 1H), 7.78 (d, J = 15.4 Hz, 1H), 7.31 (s, 2H), 7.23 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 7.0 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 7.5 Hz, 1H), 4.99 (bs, 2H), 3.92 (s, 6H), 3.77 (s, 3H).	2
Ie		*N*-(2-Amino-phenyl)-4-[3-(4-chloro-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.90 (s, 1H), AB system (δ_A = 8.33, δ_B = 8.19, J = 8.4 Hz, 4H), 8.08 (d, J = 15.4 Hz, 1H), AB system (δ_A = 8.02, δ_B = 7.60, J = 8.6 Hz, 4H), 7.83 (d, J = 15.4 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.03 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.65 (t, J = 7.5 Hz, 1H), 5.00 (bs, 2H).	2
If		*N*-(2-Amino-phenyl)-4-[3-(2,4,6-trimethoxy-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.86 (bs, 1H), 8.26-8.03 (m, 5H), 7.92 (d, J = 15.8 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 7.03 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 6.8 Hz, 1H), 6.38 (s, 2H), 4.99 (bs, 2H), 3.98 (s, 6H), 3.91 (s, 3H).	2
Ig		*N*-(2-Amino-phenyl)-4-[3-(3-cyclopropoxy-4-difluoromethoxy-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.89 (s, 1H), AB system (δ_A = 8.31, δ_B = 8.20, J = 8.1 Hz, 4H), 8.00 (d, J = 15.8 Hz, 1H), 7.83 (d, J = 15.4 Hz, 1H), ABX system (δ_A = 7.30, δ_B = 7.64, δ_X = 7.96, J = 8.4, 1.3, 0 Hz, 3H), 7.23 (d, J = 7.5 Hz, 1H), 7.17 (t, J_{HF} = 74.3 Hz, 1H), 7.04 (t, J = 7.3 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 7.3 Hz, 1H), 5.00 (bs, 2H), 4.15-4.08 (m, 1H), 0.98-0.73 (m, 4H).	2
Ih		*N*-(2-Amino-phenyl)-4-[3-(4-trifluoromethyl-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.91 (bs, 1H), 8.35 (d, J = 7.9 Hz, 2H), 8.30-8.10 (m, 5H), 7.95-7.82 (m, 3H), 7.23 (d, J = 7.9 Hz, 1H), 7.04 (t, J = 7.3 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 7.3 Hz, 1H), 5.00 (bs, 2H).	2

Cmpd	Ar	Name	Characterization	Scheme
Ii		*N*-(2-Amino-phenyl)-4-[3-(2,5-difluoro-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.90 (s, 1H), 8.34 (d, J = 7.9 Hz, 2H), 8.25-8.05 (m, 4H), 7.86 (d, J = 15.8 Hz, 1H), 751-7.38 (m, 2H), 7.23 (d, J = 7.5 Hz, 1H), 7.04 (t, J = 7.3 Hz, 1H), 6.84 (d, J = 7.9 Hz, 1H), 6.65 (t, J = 7.3 Hz, 1H), 5.00 (bs, 2H).	2
Ij		*N*-(2-Amino-phenyl)-4-[3-(3-cyclopentyloxy-4-methoxy-phenyl)-acryloyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.88 (s, 1H), AB system (δ_A = 8.29, δ_B = 8.19, J = 7.9 Hz, 4H), 7.88 (d, J = 15.8 Hz, 1H), 7.78 (d, J = 15.8 Hz, 1H), 7.55 (s, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 7.5 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 7.3 Hz, 1H), 4.99 (bs, 3H), 3.86 (s, 3H), 2.10-1.55 (m, 8H).	1

Example 11

*N-(2-Amino-phenyl)-4-[2-(3,4,5-trimethoxy-phenylcarbamoyl)-vinyl]-benzamide (**Va**)*

Step 1: Methyl 4-(2-t-butoxycarbonyl-vinyl)-benzoate (**VI**)

[0111] To a solution of anhydrous *i*-Pr_2NH (1.76 ml, 12.49 mmol) in anhydrous THF (30 ml) stirred at 0°C under nitrogen , was slowly added a solution of *n*-BuLi (5.36 ml, 13.40 mmol, 2.5 M in hexane). After 30 min, LDA was cooled to –78°C and t-butyl acetate (1.64 ml, 12.18 mmol) was added dropewise. After 30 min, a solution of methyl 4-formylbenzoate (1.00 g, 6.09 mmol) in anhydrous THF (10 ml) was slowly added. After 2 h, a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.604 g, 9.14 mmol) in anhydrous THF (10 ml) was added. Then, the temperature was allowed to warm up to room temperature overnight. A suspension appeared. The reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (AcOEt/hexane : 10/90→15/85) to give the title product **VI** (785 mg, 3.00 mmol, 49% yield) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : AB system (δ_A = 8.04, δ_B = 7.57, J = 8.4 Hz, 4H), 7.60 (d, J = 15.4 Hz, 1H), 6.46 (d, J = 15.8 Hz, 1H), 3.93 (s, 3H), 1.54 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ(ppm) : 166.72, 166.01, 142.31, 139.18, 131.33, 130.26, 127.99, 122.87, 81.11, 52.46, 28.40.

Step 2: Methyl 4-(2-carboxy-vinyl)-benzoate (**VII**)

[0112] To a stirred solution at room temperature of **VI** (745 mg, 2.84 mmol) in CH_2Cl_2 (10 ml) was added trifluoroacetic acid (6 ml, 95% in water). After 27 h, the reaction mixture was concentrated, and triturated in water. After 1 h, the suspension was filtered off, rinsed with H_2O, and dried to afford the title compound **VII** (556 mg, 2.70 mmol, 95% yield) as an off-white solid. ^1H NMR (300 MHz, DMSO-d_6) δ(ppm) : AB system (δ_A = 8.01, δ_B = 7.88, J = 8.1 Hz, 4H), 7.68 (d, J = 15.8 Hz, 1H), 6.70 (d, J = 16.3 Hz, 1H), 3.90 (s, 3H).

Method A, Step 3: Methyl 4-[2-(benzotriazol-1-yloxycarbonyl)-vinyl]-benzoate (**VIII**)

[0113] To a stirred solution at room temperature of **VII** (264 mg, 1.28 mmol) in anhydrous DMF (10 ml) under nitrogen were added Et_3N (196 µl, 1.41 mmol) and BOP reagent (680 mg, 1.1.54 mmol), respectively. After few min, a precipitate appeared. After 3 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, concentrated a little bit, and hexane was added. The suspension was filtered off and rinsed with hexane. The solid was triturated

in water, filtered off, rinsed with water, and dried to afford the title compound **VIII** (346 mg, 1.07 mmol, 84% yield) as a pale yellow solid (not stable on silica gel !). ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) : 8.56 (d, J = 8.3 Hz, 1H), 8.21-8.02 (m, 3H), 7.90-7.72 (m, 4H), 7.62 (t, J = 7.4 Hz, 1H), 3.97 (s, 3H).

Step 4: Methyl 4-[2-(3,4,5-trimethoxy-phenylcarbamoyl)-vinyl]-benzoate (**IXa**)

[0114] To a stirred suspension at room temperature of **VIII** (150 mg, 0.46 mmol) in anhydrous CH_2Cl_2 (10 ml) under nitrogen were added Et_3N (194 µl, 1.39 mmol) and 3,4,5-trimethoxyaniline (94 mg, 0.51 mmol) or $ArNH_2$ (1.1-1.2 equiv.), respectively. The reaction mixture was heated to 60°C. After 20 h, the reaction mixture was concentrated, diluted with AcOEt, and successively washed with a saturated aqueous solution of NH_4Cl, H_2O and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (AcOEt/CH_2Cl_2 : 15/85→20/80) to give the title product **IXa** (130 mg, 0.35 mmol, 75% yield) as a yellow solid. ^{1}H NMR (300 MHz, acetone-d_6) δ(ppm) : 9.42 (bs, 1H), AB system (δ_A = 8.09, δ_B = 7.78, J = 8.1 Hz, 4H), 7.75 (d, J = 15.6 Hz, 1H), 7.21 (s, 2H), 7.00 (d, J = 15.8 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 6H), 3.73 (s, 3H).

Step 5: 4-[2-(3,4,5-Trimethoxy-phenylcarbamoyl)-vinyl]-benzoate (**Xa**)

[0115] To a stirred solution at room temperature of **IXa** (125 mg, 0.34 mmol) in THF (5 ml) was added a solution of $LiOH.H_2O$ (35 mg, 0.84 mmol) in water (5 ml). After 1.5 day, the reaction mixture was concentrated, diluted with water and acidified with 1N HCl until pH 4-5 in order to get a precipitate. After stirring for 10 min, the suspension was filtered off, rinsed with water, and dried to afford the title compound **Xa** (110 mg, 0.31 mmol, 91% yield) as a pale yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 10.29 (s, 1H), AB system (δ_A = 8.04, δ_B = 7.76, J = 8.4 Hz, 4H), 7.65 (d, J = 15.8 Hz, 1H), 7.13 (s, 2H), 6.94 (d, J = 15.8 Hz, 1H), 3.81 (s, 6H), 3.67 (s, 3H).

Step 6: *N*-(2-Amino-phenyl)-4-[2-(3,4,5-trimethoxy-phenylcarbamoyl)-vinyl]-benzamide (**Va**)

[0116] To a stirred solution at room temperature of **Xa** (110 mg, 0.31 mmol) in anhydrous DMF (3 ml) under nitrogen were added Et_3N (47 µl, 0.34 mmol) and BOP reagent (163 mg, 0.37 mmol), respectively. After 30 min, a solution of 1,2-phenylenediamine (37 mg, 0.34 mmol), Et_3N (129 µl, 0.92 mmol) in anhydrous DMF (1 ml) was added dropwise. After 3 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel

(AcOEt/CH_2Cl_2 : 50/50→80/20) to afford the title compound **Va** (98 mg, 0.22 mmol, 71% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 10.27 (s, 1H), 9.76 (s, 1H), AB system (δ_A = 8.09, δ_B = 7.78, J = 7.9 Hz, 4H), 7.71 (d, J = 15.8 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.14 (s, 2H), 7.02 (t, J = 7.0 Hz, 1H), 6.95 (d, J = 15.8 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.65 (t, J = 7.5 Hz, 1H), 4.97 (bs, 2H), 3.81 (s, 6H), 3.68 (s, 3H).

Example 12

*N-(2-Amino-phenyl)-4-{2-[(pyridin-3-ylmethyl)-carbamoyl]-vinyl}-benzamide (**Vb**)*

Method B, Step 3: Methyl 4-[2-(pyridin-3-ylmethyl)-carbamoyl)-vinyl]-benzoate (**Vb**)

[0117] To a stirred solution at room temperature of **VIII** (140 mg, 0.68 mmol) in anhydrous DMF (5 ml) under nitrogen were added Et_3N (104 µl, 0.75 mmol) and BOP reagent (331 mg, 0.75 mmol), respectively. After 30 min, a solution of 3-(aminomethyl)pyridine (90 µl, 0.88 mmol) or R^1R^2NH (1.2-1.3 equiv.), Et_3N (284 µl, 2.04 mmol) in anhydrous DMF (2 ml) was added dropwise. After 4 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel (MeOH/CH_2Cl_2 : 5/95→7/93) to afford the title compound **IXb** (185 mg, 0.62 mmol, 92% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) : 8.67-8.44 (m, 2H), AB system (δ_A = 8.03, δ_B = 7.55, J = 8.4 Hz, 4H), 7.78-7.64 (m, 2H), 7.33-7.26 (m, 1H), 6.54 (d, J = 15.8 Hz, 1H), 6.38 (bs, 1H), 4.61 (d, J = 6.2 Hz, 2H), 3.92 (s, 3H).

Step 4: *N*-(2-Amino-phenyl)-4-{2-[(pyridin-3-ylmethyl)-carbamoyl]-vinyl}-benzamide (**Vb**)

[0118] The title compound **Vb** was obtained from **IXb** in two steps following the same procedure as **Example 10**, steps 5 and 6 (Scheme 3). ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.74 (s, 1H), 8.79 (t, J = 5.7 Hz, 1H), 8.58 (s, 1H), 8.52 (d, J = 4.0 Hz, 1H), 8.06 (d, J = 7.9 Hz, 2H), 7.83-7.68 (m, 3H), 7.59 (d, J = 15.8 Hz, 1H), 7.41 (dd, J = 7.9, 4.7 Hz, 1H), 7.21 (d, J = 7.9 Hz, 1H), 7.02 (t, J =7.0 Hz, 1H), 6.83 (d, J = 15.8 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 4.96 (bs, 2H), 4.48 (d, J = 5.7 Hz, 2H).

Examples 13-15

[0119] **Examples 13 to 15** (compounds **Vc-Ve**) were prepared using the same procedure as described for compound **Vb** of **Example 12** (Scheme 3).

Example 16

N-(2-Amino-phenyl)-4-[2-(2-pyridin-3-yl-ethylcarbamoyl)-vinyl]-benzamide (**Vf**)

Step 1: t-Butyl [2-(4-formyl-benzoylamino)-phenyl]-carbamate (**XI**)

[0120] To a stirred suspension at room temperature of 4-carboxybenzaldehyde (3.00 g, 19.98 mmol) in anhydrous CH_2Cl_2 (10 ml) under nitrogen were added thionyl chloride (2.19 ml, 29.97 mmol) and anhydrous DMF (387 µl, 5.00 mmol), respectively. The reaction mixture was refluxed for 5h. Then, the reaction mixture was allowed to cool to room temperature, concentrated, and diluted with anhydrous CH_2Cl_2 (20 ml) under nitrogen. This solution was canulated into a cooled mixture at –20°C of t-butyl (2-amino-phenyl)-carbamic ester (4.575 g, 21.98 mmol), Et_3N (8.36 ml, 59.95 mmol) in anhydrous CH_2Cl_2 (50 ml) under nitrogen. After 1h, the reaction mixture was allowed to warm up to room temperature. After 1h, it was poured into a saturated aqueous solution of NH_4Cl, and extracted with CH_2Cl_2. The combined organic layer was successively dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane : 30/70→40/60) to afford the title compound **XI** (4.80 g, 14.11 mmol, 71% yield) as a pale yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : 10.11 (s, 1H), 9.58 (bs, 1H), AB system (δ_A = 8.14, δ_B = 7.99, J = 8.1 Hz, 4H), 7.89 (d, J = 7.9 Hz, 1H), 7.35-7.10 (m, 3H), 6.75 (s, 1H), 1.53 (s, 9H).

Step 2: Methyl 3-[4-(2-t-butoxycarbonylamino-phenylcarbamoyl)-phenyl]-acrylate (**XII**)

[0121] A stirred suspension of compound **XI** (500 mg, 1.47 mmol), methyl (triphenyl-phosphoranylidene)acetate (590 mg, 1.76 mmol) in anhydrous toluene (20 ml) was heated at 90°C under nitrogen. After 2 days, the reaction mixture was concentrated and directly purified by flash chromatography on silica gel (AcOEt/hexane : 30/70→40/60) to afford the title compound **XII** (568 mg, 1.43 mmol, 97% yield) as a pale yellow foam. ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : 9.32 (bs, 1H), AB system (δ_A = 7.99, δ_B = 7.62, J = 8.4 Hz, 4H), 7.87 (d, J = 7.9 Hz, 1H), 7.73 (d, J = 15.8 Hz, 1H), 7.32-7.13 (m, 3H), 6.69 (bs, 1H), 6.53 (d, J = 16.3 Hz, 1H), 3.83 (s, 3H), 1.53 (s, 9H).

Step 3: 3-[4-(2-t-Butoxycarbonylamino-phenylcarbamoyl)-phenyl]-acrylic acid (**XIII**)

[0122] To a stirred solution at room temperature of compound **XII** (560 mg, 1.41 mmol) in THF (20 ml) was added a solution of $LiOH.H_2O$ (148 mg, 3.53 mmol) in water (20 ml). After 23 h, the reaction mixture was concentrated, diluted with water and acidified with 1N HCl until pH 4-5 in order to get a white precipitate. After stirring for 15 min, the suspension was filtered off, rinsed with water, and dried to afford the title compound **XIII** (495 mg, 1.29 mmol, 92% yield) as a white solid. ^1H NMR

(300 MHz, DMSO-d_6) δ(ppm) :. 9.92 (s, 1H), 8.72 (bs, 1H), AB system (δ_A = 8.02, δ_B = 7.90, J = 7.9 Hz, 4H), 7.69 (d, J = 16.3 Hz, 1H), 7.62-7.53 (m, 2H), 7.30-7.13 (m, 2H), 6.72 (d, J = 16.3 Hz, 1H), 1.48 (s, 9H)

Step 4: *t*-Butyl (2-{4-[2-(2-pyridin-3-yl-ethylcarbamoyl)-vinyl]-benzoylamino}-phenyl)-carbamate (**XIVf**)

[0123] To a stirred solution at room temperature of compound **XIII** (80 mg, 0.21 mmol) in anhydrous DMF (3 ml) under nitrogen were added Et_3N (35 µl, 0.25 mmol) and BOP reagent (102 mg, 0.23 mmol), respectively. After 30 min, a solution of 3-(2-aminoethyl)pyridine (51 mg, 0.42 mmol) or RXH (1.5-2.0 equiv.), Et_3N (87 µl, 0.63 mmol) in anhydrous DMF (1 ml) was added dropwise. After 3-5 h, the reaction mixture was poured into a saturated aqueous solution of NH_4Cl, and diluted with AcOEt. After separation, the organic layer was successively washed with sat NH_4Cl, H_2O and brine, dried over $MgSO_4$, filtered, and concentrated to afford the title compound **XIVf**. It was used in the next step without further purification.

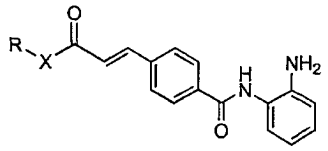
Step 5: *N*-(2-Amino-phenyl)-4-[2-(2-pyridin-3-yl-ethylcarbamoyl)-vinyl]-benzamide (**Vf**)

[0124] To a stirred solution at room temperature of **XIVf** in CH_2Cl_2 (15 ml) was added trifluoroacetic acid (2 ml, 95% in water). After 18 h, the reaction mixture was concentrated, dissolved in water, and neutralized with a saturated aqueous solution of $NaHCO_3$ until a pH = 7. A pale yellow precipitate appeared. After few minutes, the suspension was filtered off, rinsed with H_2O, and dried to afford the title compound **Vf** (69 mg, 0.18 mmol, 85% yield for two steps) as a pale yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.72 (s, 1H), 8.53-8.41 (m, 2H), 8.29 (t, J = 5.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 2H), 7.80-7.63 (m, 3H), 7.51 (d, J = 15.8 Hz, 1H), 7.37 (dd, J = 7.5, 4.8 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.76 (d, J = 15.8 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 4.95 (bs, 2H), 3.51 (dd, J = 6.8 Hz, 2H), 2.86 (t, J = 6.8 Hz, 2H).

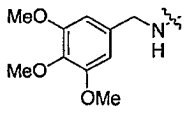
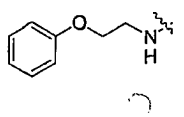
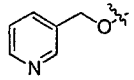
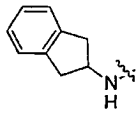
Examples 17-26

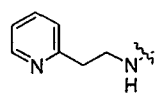
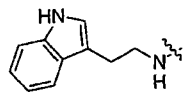
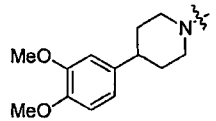
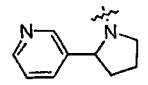
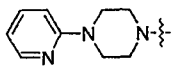
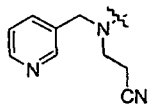
[0125] **Examples 17 to 26** (compounds **Vg-Vp**) were prepared using the same procedure as described for compound **Vf** of **Example 16** (Scheme 4).

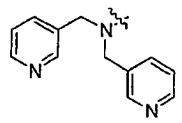
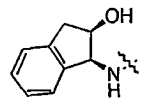
Table 3



V

Cmpd	Rx-	Name	Characterization	Scheme
Vc		*N*-(2-Amino-phenyl)-4-[2-(3,4,5-trimethoxy-benzylcarbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (s, 1H), 8.64 (t, J = 5.7 Hz, 1H), AB system (δ_A = 8.06, δ_B = 7.74, J = 8.1 Hz, 4H), 7.58 (d, J = 15.8 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 7.3 Hz, 1H), 6.85 (d, J = 15.8 Hz, 1H), 6.82 (d, J = 7.0 Hz, 1H), 6.68 (s, 2H), 6.64 (t, J = 7.5 Hz, 1H), 4.95 (bs, 2H),), 4.39 (d, J = 5.7 Hz, 2H), 3.81 (s, 6H), 3.67 (s, 3H).	3
Vd		*N*-(2-Amino-phenyl)-4-[2-(2-phenoxy-ethylcarbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (s, 1H), 8.48 (t, J = 5.3 Hz, 1H), AB system (δ_A = 8.06, δ_B = 7.74, J = 8.3 Hz, 4H), 7.56 (d, J = 15.8 Hz, 1H), 7.34 (t, J = 7.9 Hz, 2H), 7.21 (d, J = 7.5 Hz, 1H), 7.10-9.90 (m, 4H), 6.85 (d, J = 15.4 Hz, 1H), 6.82 (d, J = 7.0 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 4.95 (bs, 2H),), 4.10 (t, J = 5.3 Hz, 2H), 3.62 (quadruplet, J = 5.3 Hz, 2H).	3
Ve		*N*-(2-Amino-phenyl)-4-(3-morpholin-4-yl-3-oxo-propenyl)-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.75 (s, 1H), AB system (δ_A = 8.05, δ_B = 7.90, J = 8.1 Hz, 4H), 7.61 (d, J = 15.4 Hz, 1H), 7.43 (d, J = 15.4 Hz, 1H),), 7.20 (d, J = 7.9 Hz, 1H), 7.02 (t, J =7.0 Hz, 1H), 6.82 (d, J = 7.9 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 4.95 (bs, 2H), 3.90-3.55 (m, 8H).	3
Vg		Pyridin-3-ylmethyl 3-[4-(2-amino-phenylcarbamoyl)-phenyl]-acrylic ester	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.75 (s, 1H), 8.72 (s, 1H), 8.61 (bs, 1H), AB system (δ_A = 8.05, δ_B = 7.92, J = 7.5 Hz, 4H, included 1H), 7.82 (d, J = 16.3 Hz, 1H), 7.55-7.43 (m, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.01 (t, J =7.3 Hz, 1H), 6.88 (d, J = 15.8 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.63 (t, J = 7.0 Hz, 1H), 5.33 (s, 2H), 4.95 (bs, 2H).	4
Vh		*N*-(2-Amino-phenyl)-4-[2-(indan-2-ylcarbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.71 (s, 1H), 8.51 (d, J = 7.0 Hz, 1H), AB system (δ_A = 8.04, δ_B = 7.71, J = 8.4 Hz, 4H), 7.54 (d, J = 15.8 Hz, 1H), 7.36-7.14 (m, 5H), 7.01 (t, J = 7.3 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.77 (d, J = 16.3 Hz, 1H), 6.63 (t, J = 7.5 Hz, 1H), 4.94 (bs, 2H), 4.71-4.57 (m, 1H), 3.28 (dd, J = 16.0, 7.3 Hz, 2H), 2.87 (dd, J = 16.3, 5.3 Hz, 2H).	4

Cmpd	Rx-	Name	Characterization	Scheme
Vi		*N*-(2-Amino-phenyl)-4-[2-(2-pyridin-2-yl-ethylcarbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.72 (s, 1H), 8.56 (d, J = 4.4 Hz, 1H), 8.28 (t, J = 5.5 Hz, 1H), 8.05 (d, J = 8.4 Hz, 2H), 7.82-7.66 (m, 3H), 7.52 (d, J = 15.8 Hz, 1H), 7.40-7.15 (m, 3H), 7.02 (t, J = 7.3 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.77 (d, J = 15.8 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 4.95 (s, 2H), 3.61 (quadruplet, J = 6.6 Hz, 2H), 2.99 (t, J = 7.2 Hz, 2H).	4
Vj		*N*-(2-Amino-phenyl)-4-{2-[2-(1*H*-indol-3-yl)-ethylcarbamoyl]-vinyl}-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm)** : 10.85 (bs, 1H), 9.73 (bs, 1H), 8.31 (t, J = 5.6 Hz, 1H), AB system (δ_A = 8.06, δ_B = 7.73, J = 8.0 Hz, 4H), 7.61 (d, J = 9.0 Hz, 1H), 7.55 (d, J = 15.0 Hz, 1H), 7.39 (d, J = 9.0 Hz, 1H), 7.28-7.15 (m, 2H), 7.11 (t, J = 7.5 Hz, 1H),7.03 (d, J = 9.0 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.81 (d, J = 15.0 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 4.94 (s, 2H), 3.54 (quadruplet, J = 6.7 Hz, 2H), 2.94 (t, J = 6.8 Hz, 2H).	4
Vk		*N*-(2-Amino-phenyl)-4-{3-[4-(3,4-dimethoxy-phenyl)-piperidin-1-yl]-3-oxo-propenyl}-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.74 (s, 1H), AB system (δ_A = 8.05, δ_B = 7.91, J = 8.1 Hz, 4H), 7.60 (d, J = 15.8 Hz, 1H), 7.49 (d, J = 15.4 Hz, 1H),), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (t, J =7.0 Hz, 1H), 6.95-6.75 (m, 4H), 6.64 (t, J = 7.3 Hz, 1H), 4.95 (bs, 2H), 4.70 (bd, J = 11.9 Hz, 1H), 4.50 (bd, J = 12.3 Hz, 1H), 3.79 and 3.75 (2s, 6H), 3.22 (bt, J = 12.3 Hz, 1H), 2.88-2.70 (m, 2H), 1.96-1.80 (m, 2H), 1.75-1.47 (m, 2H).	4
Vl		(rac)-*N*-(2-Amino-phenyl)-4-[3-oxo-3-(2-pyridin-3-yl-pyrrolidin-1-yl)-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** mixture of rotamers, 9.75 and 9.67 (2s, 1H), 8.55-8.45 (m, 2H), 8.05-6.58 (m, 12H), 5.60-5.55 and 5.25-5.20 (2m, 1H), 5.00-4.90 (m, 2H), 4.15-3.65 (m ,2H), 2.50-2.28 (m, 1H), 2.10-1.75 (m, 3H).	4
Vm		*N*-(2-Amino-phenyl)-4-[3-oxo-3-(4-pyridin-2-yl-piperazin-1-yl)-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.75 (s, 1H), 8.18 (d, J = 3.5 Hz, 1H), AB system (δ_A = 8.06, δ_B = 7.92, J = 8.1 Hz, 4H), 7.70-7.55 (m, 2H), 7.49 (d, J = 15.4 Hz, 1H),), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (t, J =7.5 Hz, 1H), 6.92 (d, J = 8.8 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.72 (t, J = 6.2 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 4.95 (bs, 2H), 4.00-3.50 (m, 8H).	4
Vn		*N*-(2-Amino-phenyl)-4-{2-[(2-cyano-ethyl)-pyridin-3-ylmethyl-carbamoyl]-vinyl}-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** mixture of rotamers, 9.75 and 9.72 (2s, 1H), 8.65-8.45 (m, 2H), 8.15-7.80 (m, 4H), 7.78-7.62 (m, 2H), 7.55-7.35 (m, 2H), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.64 (t, J = 7.3 Hz, 1H), 5.02 (bs, 1H), 4.95 (bs, 2H), 4.74 (bs, 1H), 3.93 (bs, 1H), 3.71 (t, J = 6.2 Hz, 1H),), 2.92 (t, J = 6.2 Hz, 1H),), 2.86 (t, J = 6.2 Hz, 1H).	4

Cmpd	Rx-	Name	Characterization	Scheme
Vo		*N*-(2-Amino-phenyl)-4-[2-(bis-pyridin-3-ylmethyl-carbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (s, 1H), 8.60-8.45 (m, 4H), AB system (δ_A = 8.03, δ_B = 7.87, J = 8.4 Hz, 4H), 7.80-7.63 (m,3H), 7.49 (d, J = 15.4 Hz, 1H), 7.45-7.32 (m, 2H), 7.20 (d, J = 7.5 Hz, 1H),7.01 (t, J = 7.0 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.63 (t, J = 7.3 Hz, 1H), 4.97 and 4.95 (2s, 4H), 4.70 (s, 2H).	4
Vp		(-)-(1S,2R)-*N*-(2-Amino-phenyl)-4-[2-(2-hydroxy-indan-1-ylcarbamoyl)-vinyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (s, 1H), 8.26 (d, J = 8.8 Hz, 1H), AB system (δ_A = 8.07, δ_B = 7.75, J = 8.1 Hz, 4H), 7.62 (d, J = 15.4 Hz, 1H), 7.35-7.15 (m, 5H), 7.13 (d, J = 15.8 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.64 (t, J = 7.5 Hz, 1H), 5.39 (dd, J = 8.4, 4.8 Hz, 1H), 5.19 (d, J = 4.0 Hz, 1H), 4.97 (bs, 2H), 4.58-4.46 (m, 1H), 3.14 (dd, J = 16.3, 4.8 Hz, 1H), 2.88 (d, J = 15.8, 5.3 Hz, 1H).	4

Example 27

*N-(2-Amino-phenyl)-4-[3-(3-cyclopentyloxy-4-methoxy-phenylamino)-propenyl]-benzamide (**XVa**)*

Step 1: *t*-Butyl {2-[4-(3-oxo-propenyl)-benzoylamino]-phenyl}-carbamate (**XVI**)

[0126] A stirred suspension of compound **XI** (4.00 g, 11.75 mmol), (triphenylphosphoranylidene)-acetaldehyde (3.60 g, 11.83 mmol) in anhydrous toluene (100 ml) was heated at 80°C under nitrogen. After 2 days, the reaction mixture was concentrated and directly purified by flash chromatography on silica gel (AcOEt/hexane : 30/70) to afford the title compound **XVI** (3.70 g, 10.10 mmol, 86% yield) as a yellow sticky solid (slightly contaminated with the diene). ^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) : 9.75 (d, J = 7.8 Hz, 1H), 9.49 (bs, 1H), AB system (δ_A = 8.03, δ_B = 7.65, J = 8.4 Hz, 4H), 7.85-7.72 (m, 1H), 7.52 (d, J = 15.6 Hz, 1H), 7.33-7.05 (m, 3H), 7.05-6.90 (m, 1H), 6.78 (dd, J = 15.6, 7.8 Hz, 1H), 1.53 (s, 9H).

Step 2: *t*-Butyl {2-[4-[3-(3-cyclopentyloxy-4-methoxy-phenylamino)-propenyl]-benzoylamino}-phenyl}-carbamate (**XVIIa**)

[0127] To a stirred solution at room temperature of compound **XVI** (210 mg, 0.57 mmol), 3-cyclopentyloxy-4-methoxy-aniline (125 mg, 0.60 mmol) or $ArNH_2$ (1.05-1.2 equiv.) in anhydrous THF (7 ml) under nitrogen were added dibutyltin dichloride (3.5 mg, 0.01 mmol). After 10 min, phenylsilane (78 µl, 0.63 mmol) was added dropwise. After 3 days, the reaction mixture was concentrated and directly purified by flash chromatography on silica gel (AcOEt/hexane : 30/70→50/50) to afford the title compound **XVIIa** as a yellow sticky oil.

Step 3: *N*-(2-Amino-phenyl)-4-[3-(3-cyclopentyloxy-4-methoxy-phenylamino)-propenyl]-benzamide (**XVa**)

[0128] To a stirred solution at room temperature of **XVIIa** in CH_2Cl_2 (30 ml) was added trifluoroacetic acid (5 ml, 95% in water). After 16 h, the reaction mixture was concentrated, dissolved in water, and basified with a aqueous solution of NaOH (1N) until a pH = 8. A beige precipitate appeared. After 15 min, the suspension was filtered off, rinsed with H_2O, and air-dried. The crude product was purified by flash chromatography on silica gel (AcOEt/CH_2Cl_2 : 15/85→20/80 + ε NH_4OH) to afford the title compound **XVa** (145 mg, 0.32 mmol, 55% yield for two steps) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : mixture of rotamers, 9.67 and 9.63 (2s, 1H), 7.98 (d, J = 7.9 Hz, 2H), 7.57 and 7.51 (2d, J = 7.9 Hz, 2H), 7.20 (d, J = 7.9 Hz, 1H), 7.01 (t, J = 7.7 Hz, 1H), 6.82 (d, J = 7.9 Hz, 1H), 6.77-6.67 (m, 2H), 6.63 (t, J = 7.5 Hz, 1H), 6.54 (dt, J = 16.3, 5.2 Hz, 1H), 6.35 and 6.30 (2d, J = 2.0 Hz, 1H), 6.15 and 6.06 (2dd, J = 8.6, 2.0 Hz, 1H), 5.98 and

5.57 (2t, J = 5.5 Hz, 1H), 4.92 (bs, 2H), 4.78-4.63 (m, 1H), 4.32 and 3.87 (2d, J = 5.7 Hz, 2H), 3.65 and 3.62 (2s, 3H), 1.95-1.45 (m, 8H).

Examples 28-32

[0129] **Examples 28 to 32** (compounds **XVb-XVf**) were prepared using the same procedure as described for compound **XVa** of **Example 27** (Scheme 5).

Example 33

*N-(2-Amino-phenyl)-4-[3-(4-tolyl-sulfonylamino)-propenyl]-benzamide (**XVg**)*

Step 1: *t*-Butyl {2-[4-(3-hydroxy-propenyl)-benzoylamino]-phenyl}-carbamate (**XVIII**)

[0130] To a stirred solution of compound **XVI** (1.00 g, 2.79 mmol) in ethanol (15 ml) under nitrogen was added sodium borohydride (110 mg, 2.73 mmol). After 5 min, the reaction mixture was quenched with water and diluted with AcOEt. After separation the organic layer was successively washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane : 40/60) to afford the title compound **XVIII** (910 mg, 2.29 mmol, 82% yield) as a pale yellow solid (slightly contaminated with the diene). ^1H NMR (300 MHz, $CDCl_3$) δ(ppm) : 9.20 (s, 1H), 7.90 (d, J = 7.8 Hz, 2H), 7.75 (d, J = 7.5 Hz, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.32-7.08 (m, 3H), 6.94 (s, 1H), 6.65 (d, J = 15.9 Hz, 1H), 6.45 (td, J = 15.9, 5.4 Hz, 1H), 4.35 (d, J = 5.4 Hz, 2H), 1.92 (s, 1H), 1.51 (s, 9H).

Step 2: *t*-butyl (2-{4-[3-(4-tolyl-sulfonylamino)-propenyl]-benzoylamino}-phenyl)-carbamate (**XVIIg**)

[0131] To a stirred solution of *N*-Boc-4-tolylsulfonamide (221 mg, 0.81 mmol) and PPh_3 (427 mg, 1.63 mmol) in anhydrous THF (4 ml) under nitrogen was successively added a solution of compound **XVIII** (200 mg, 0.54 mmol) in anhydrous THF (1 ml) and diethyl azodicarboxylate (DEAD) (214 µl, 1.36 mmol). After 16 h, the reaction mixture was quenched with water and diluted with AcOEt. After separation the organic layer was successively washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane : 40/60) to afford the title compound **XVIIg** (337 mg).

Step 3: *N*-(2-Amino-phenyl)-4-[3-(4-tolyl-sulfonylamino)-propenyl]-benzamide (**XVg**)

[0132] To a stirred solution at room temperature of **XVIIg** in CH_2Cl_2 (20 ml) was added trifluoroacetic acid (2 ml, 95% in water). After 16 h, the reaction mixture was concentrated, dissolved in water, and basified with a aqueous saturated solution of $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was successively washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude residue was solubilized with a minimum of a mixture of

AcOEt/MeOH (95/5) and coprecipitated with hexane. An off-white precipitate appeared. After few minutes, the suspension was filtered off, rinsed with hexane and dried to give the title compound **XVg** (173 mg, 0.41 mmol, 76% yield for two steps) as an off-white solid. ^{1}H NMR: (300 MHz, DMSO-d_6) δ (ppm) : 9.64 (s, 1H), AB system (δ_A = 7.93, δ_B = 7.72, J = 8.4 Hz, 4H), 7.84 (s, 1H), 7.41 (t, J = 8.4 Hz, 4H), 7.16 (d, J = 7.8 Hz, 1H), 6.97 (t, J = 7.5 Hz, 1H), 6.77 (d, J = 7.5 Hz, 1H), 6.59 (t, J = 7.8 Hz, 1H), 6.52 (d, J = 15.6 Hz, 1H), 6.21 (dt, J = 15.6, 5.7 Hz, 1H), 4.89 (s, 2H), 3.60 (bs, 2H), 2.08 (s, 3H).

Example 34

*N-(2-Amino-phenyl)-4-{3-[(pyridin-3-ylmethyl)-amino]-propenyl}-benzamide (**XVh**)*

Step 1: Methyl 4-(3-oxo-propenyl)-benzoate (**XIX**)

[0133] Method A: A stirred suspension of compound methyl 4-formylbenzoate (4.00 g, 24.37 mmol), (triphenylphosphoranylidene)-acetaldehyde (7.56 g, 24.85 mmol) in anhydrous toluene (100 ml) was heated at 80-90°C under nitrogen. After 1 day, the reaction mixture was concentrated and directly purified by flash chromatography on silica gel (AcOEt/hexane : 20/80□30/70) to afford the title compound **XIX** (2.52 g, 13.25 mmol, 54% yield) as a pale yellow solid (slightly contaminated with the diene). ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) : 9.76 (d, J = 7.3 Hz, 1H), AB system (δ_A = 8.11, δ_B = 7.64, J = 8.1 Hz, 4H), 7.51 (d, J = 15.6 Hz, 1H), 6.79 (dd, J = 15.8, 7.6 Hz, 1H), 3.95 (s, 3H).

[0134] Method B: To a vigorously stirred emulsion at room temperature of TDA-1 (6.278 g, 19.41 mmol) and an aqueous solution of 10% of potassium carbonate (100 ml) in CH_2Cl_2 (100 ml) were added (1,3-dioxolan-2-yl)methyltriphenylphosphonium bromide (10 g, 23.29 mmol) and methyl 4-formylbenzoate (3.187 g, 19.41 mmol), respectively. After stirring for 18 h, the reaction mixture was extracted with CH_2Cl_2 and the combined organic layer was concentrated. Then, an aqueous solution of 10% HCl (100 ml) was added and the mixture stirred overnight at room temperature. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The combined organic layer was successively dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane: 20/80□30/70) and triturated in AcOEt/hexane, to afford the title compound **XIX** (2.50 g, 13.14 mmol, 68% yield) as a crystalline solid (pure *trans* geometry and free of diene).

Step 2: Methyl 4-{3-[(pyridin-3-ylmethyl)-amino]-propenyl}-benzoate (**XXh**)

[0135] A solution at room temperature of compound **XIX** (300 mg, 1.58 mmol) and 3-(aminomethyl)pyridine (193 µl, 0.60 mmol) or RNH_2 (1.1-1.2 equiv.) in anhydrous dichloromethane

(15 ml) under nitrogen was stirred for 1 h, and sodium triacetoxyborohydride (401 mg, 1.89 mmol) was added. After 64 h, the reaction mixture was quenched with an aqueous solution of K_2CO_3 (10%) and extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude residue was then purified by flash chromatography on silica gel ($MeOH/CH_2Cl_2$: 5/95 + ε NH_4OH) to afford the title compound **XXh** (188 mg, 0.66 mmol, 42% yield) as a dark yellow oil.

Step 3: 4-[3-(*tert*-Butoxycarbonyl-pyridin-3-ylmethyl-amino)-propenyl]-benzoic acid (**XXIh**)

[0136] To a stirred solution at room temperature of **XXh** (187 mg, 0.66 mmol) in 1,4-dioxane (7 ml) were added $(Boc)_2O$ (173 mg, 0.80 mmol) and an aqueous solution of NaOH (3.3 ml, 1N), respectively. After 24 h, the reaction mixture was concentrated, diluted in water, and neutralized (pH = 6-7) with a aqueous solution of HCl (1N). The resulting pale yellow suspension was extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$, filtered and concentrated to afford the title compound **XXIh** (160 mg, 0.43 mmol, 66% yield) as a yellow solid.

Step 4: *t*-Butyl [3-[4-(2-amino-phenylcarbamoyl)-phenyl]-allyl}-pyridin-3-ylmethyl-carbamate (**XXIIh**)

[0137] The title compound **XXIIh** (Example 34) was obtained from **XXIh** as pale- yellow foam in one step following the same procedure as in Example 11, step 6.

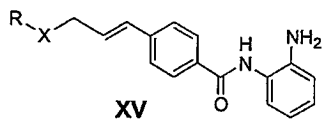
Step 5: *N*-(2-Amino-phenyl)-4-{3-[(pyridin-3-ylmethyl)-amino]-propenyl}-benzamide (**XVh**)

[0138] To a stirred solution at room temperature of **XXIIh** (77 mg, 0.17 mmol) in dichloromethane (10 ml) was added TFA (2 ml, 95% in water). After 4.5 h, the reaction mixture was concentrated, diluted in water, basified (pH = 9) with a aqueous solution of NaOH (1N), and extracted with dichloromethane. The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was then purified by flash chromatography on silica gel ($MeOH/CH_2Cl_2$: 10/90 + ε NH_4OH) to afford the title compound **XVh** (35 mg, 0.10 mmol, 58% yield) as a yellow powder. ^1H NMR: (400 MHz, DMSO-d_6) δ (ppm) : 9.64 (s, 1H), 8.55 (s, 1H), 8.44 (d, J = 3.9 Hz, 1H), AB system (δ_A = 7.94, δ_B = 7.55, J = 8.0 Hz, 4H), 7.78 (d, J = 7.4 Hz, 1H), 7.36 (dd, J = 7.0, 5.1 Hz, 1H), 7.16 (d, J = 7.4 Hz, 1H), 6.98 (t, J = 7.4 Hz, 1H), 6.79 (d, J = 7.8 Hz, 1H), 6.65-6.55 (m ,2H), 6.51 (dt, J = 16.0, 5.9 Hz, 1H), 4.93 (bs, 2H), 3.77 (s, 2H).

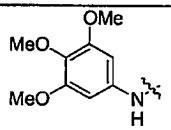
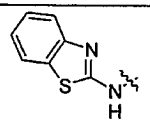
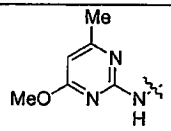
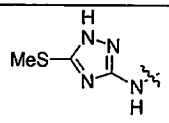
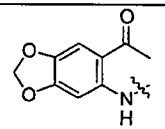
Examples 35-36

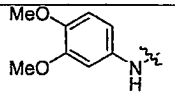
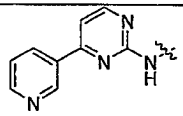
[0139] **Examples 35 to 36** (compounds **XVi-XVj**) were prepared using the same procedure as described for compound **XVh** of **Example 34** (Scheme 7, Pathway B).

Table 4



XV

Cmpd	Rx-	Name	Characterization	Scheme
XVb		*N*-(2-Amino-phenyl)-4-[3-(3,4,5-trimethoxy-phenylamino)-propenyl]-benzamide	**^{1}H NMR (300 MHz, $CDCl_3$) δ(ppm) :** 7.94 (m, 1H), AB system (δ_A = 7.85, δ_B = 7.45, J = 8.4 Hz, 4H), 7.33 (d, J = 8.1 Hz, 1H), 7.09 (t, J = 7.2 Hz, 1H), 6.90-6.78 (m, 2H), 6.69 (d, J = 15.9 Hz, 1H), 6.45 (td, J = 15.9, 5.7 Hz, 1H), 6.95 (s, 2H), 3.96 (d, J = 5.7 Hz, 2H), 3.82 (s, 6H), 3.77 (s, 3H).	5
XVc		*N*-(2-Amino-phenyl)-4-[3-(benzothiazol-2-ylamino)-propenyl]-benzamide	**^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) :** 9.65 (bs, 1H), 8.30 (t, J = 4.9 Hz, 1H), AB system (δ_A = 7.96, δ_B = 7.58, J = 7.8 Hz, 4H), 7.69 (d, J = 7.3 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 6.8 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.71 (d, J = 16.1 Hz, 1H), 6.62-6.51 (m, 2H), 4.89 (bs, 2H), 4.26-4.19 (m ,2H).	5
XVd		*N*-(2-Amino-phenyl)-4-[3-(4-methoxy-6-methyl-pyrimidin-2-ylamino)-propenyl]-benzamide	**^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) :** mixture of rotamers, 9.63 (bs, 1H), AB system (δ_A = 7.93, δ_B = 7.53, J = 7.5 Hz, 4H), 7.35-7.05 (m, 2H), 6.96 (t, J = 6.5 Hz, 1H), 6.77 (d, J = 7.9 Hz, 1H), 6.65-6.40 (m, 3H), 5.91 (bs, 1H), 4.15-4.03 (m, 2H), 3.80 (bs, 3H), 2.16 (bs, 3H).	5
XVe		*N*-(2-Amino-phenyl)-4-[3-(5-methylsulfanyl-1H-[1,2,4]triazol-3-ylamino)-propenyl]-benzamide	**^{1}H NMR (500 MHz, DMSO-d_6) δ(ppm) :** 12.16 (bs, 1H), 9.64 (s, 1H), 8.00-7.90 (m, 2H), 7.60-7.50 (m, 2H), 7.20-7.13 (m, 1H), 7.02-6.85 (m, 2H), 6.82-6.75 (m, 1H), 6.67-6.55 (m, 2H), 6.55-6.42 (m, 1H), 4.88 (bs, 2H), 3.93 (bs, 2H), 2.42 (s, 3H).	5
XVf		4-[3-(6-Acetyl-benzo[1,3]dioxol-5-ylamino)-propenyl]-*N*-(2-amino-phenyl)-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) :** 9.64 (s, 1H), 9.47 (t, J = 6.0 Hz, 1H), 7.92 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.32 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 6.95 (td, J = 8.4, 1.6 Hz, 1H), 6.76 (dd, J = 8.4, 1.6 Hz, 1H), 6.63 (d, J = 16.0 Hz, 1H), 6.58 (td, J = 8.4, 1.6 Hz, 1H), 6.54 (dt, J = 16.0, 5.6 Hz, 1H), 6.46 (s, 1H), 5.97 (s, 2H), 4.88 (s, 2H), 4.07 (t, J = 5.6 Hz, 2H), 2.45 (s, 3H).	5

Cmpd	Rx-	Name	Characterization	Scheme
XVg		*N*-(2-Amino-phenyl)-4-[3-(3,4-dimethoxy-phenylamino)-propenyl]-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) :** 9.67 (s,1H), AB system (δ_A = 7.96, δ_B = 7.56, J = 8.2 Hz, 4H), 7.18 (d, J = 6.7 Hz, 1H), 6.99 (td, J = 7.6, 1.6 Hz, 1H), 6.80 (dd, J = 8.0, 1.4 Hz, 1H), ABX system (δ_A = 6.74, δ_B = 6.13, δ_X = 6.37, J_{AB} = 8.5 Hz, J_{BX} = 2.6 Hz, J_{AX} = 0 Hz, 3H), 6.71 (d, J = 16.2 Hz, 1H), 6.62 (td, J = 7.5, 1.4 Hz, 1H), 6.52 (dt, J = 16.0, 5.5 Hz, 1H), 5.59 (t, J = 5.9 Hz, 1H), 4.92 (s, 2H), 3.88 (t, J = 4.9 Hz, 2H), 3.72 (s, 3H), 3.65 (s, 3H).	7 (Pathway B)
XVh		*N*-(2-Amino-phenyl)-4-[3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-propenyl]-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) :** 9.65 (s, 1H), 9.31 (s, 1H), 8.71 (dd, J = 4.8, 1.7 Hz, 1H), 8.53-8.42 (m, 2H), 7.95 (d, J = 8.2 Hz, 2H), 7.67 (t, J = 5.9 Hz, 1H), 7.60-7.52 (m, 3H), 7.30 (d, J = 5.1 Hz, 1H), 7.17 (d, J = 6.7 Hz, 1H), 6.99 (td, J = 7.6, 1.6 Hz, 1H), 6.79 (dd, J = 8.0, 1.4 Hz, 1H), 6.69 (bd, J = 16.0 Hz, 1H), 6.64-6.53 (m, 2H), 4.92 (s, 2H), 4.32-4.20 (m, 2H).	7 (Pathway B)

Example 37

*N-(2-Amino-phenyl)-4-(3-oxo-3-phenyl-propenyl)-benzamide (**XXIVa**)*

Step 1: 4-(3-Oxo-3-phenyl-propenyl)-benzoic acid (**XIIIa**)

[0140] To a stirred suspension at room temperature of 4-formylbenzoic acid (2.58 g, 17 mmol) and acetophenone (2.0 ml, 17 mmol) or acetophenone derivatives (1.0-1.1 equiv.) in MeOH (100 ml) was added a solution of NaOH (34 ml, 1N in H_2O). After 16 h, the reaction mixture was acidified with conc. HCl (pH =1-2), filtered off, rinsed with H_2O and dried to afford the title compound **XXIIIa** (3.73 g, 14.6 mmol, 86% yield) as a yellow solid.

Step 2: *N*-(2-Amino-phenyl)-4-(3-oxo-3-phenyl-propenyl)-benzamide (**XXIVa**)

[0141] The title compound **XXIVa** was obtained from **XXIIIa** in one step following the same procedure as **Example 1**, step 2 (Scheme 1). ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.77 (s, 1H); 8.21 (d, J = 7.0 Hz, 2H); 8.06 (m, 5H), 7.82 (d, J = 15.4 Hz, 1H), 7.71 (t, J = 7.3 Hz, 1H), 7.60 (t, J = 7.3 Hz, 2H), 7.18 (d, J = 7.9 Hz, 1H), 6.99 (t, J = 7.0 Hz, 1H), 6.80 (d, J = 7.5 Hz, 1H), 6.61 (t, J = 7.3 Hz, 1H), 4.95 (bs, 2H).

Examples 38-41

[0142] **Examples 38 to 41** (compounds **XXIVb-XXIVe**) were prepared using the same procedure as described for compound **XXIVa** of **Example 37** (Scheme 8).

Example 42

*N-(2-Amino-phenyl)-4-[3-(4-morpholin-4-yl-phenyl)-3-oxo-propenyl]-benzamide (**XXIVf**)*

Step 1: *t*-Butyl (2-{4-[3-(4-morpholin-4-yl-phenyl)-3-oxo-propenyl]-benzoylamino}-phenyl)-carbamate (**XXVf**)

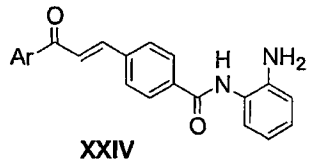
[0143] To a stirred solution at room temperature of **XI** (210 mg, 0.62 mmol), 4'-morpholino acetophenone (227 mg, 1.11 mmol) or acetophenone derivative (1.5-2.0 equiv.) in MeOH (10 ml) was added a solution of NaOH (1.9 ml, 1N in H_2O). A precipitate appeared. After 3 days, the reaction mixture was filtered off, rinsed with MeOH, air-dried and dried under vacuum to afford the title compound **XXVf** (295 mg, 0.56 mmol, 90% yield) as a yellow solid.

Step 2: *N*-(2-Amino-phenyl)-4-[3-(4-morpholin-4-yl-phenyl)-3-oxo-propenyl]-benzamide (**XXIVf**)

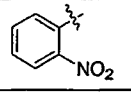
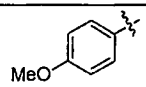
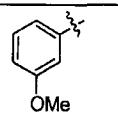
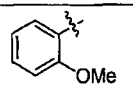
[0144] To a stirred solution at room temperature of **XXVf** (285 mg, 0.54 mmol) in CH_2Cl_2 (10 ml) was added trifluoroacetic acid (2 ml, 95% in water). After 17 h, the reaction mixture was concentrated, diluted with AcOEt, successively washed with sat $NaHCO_3$, H_2O, sat NH_4Cl, H_2O and

brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was co-precipitated in a mixture of AcOEt/hexane and triturated. After few hours, the suspension was filtered off, rinsed with hexane and dried to afford the title compound **XXIVf** (210 mg, 0.49 mmol, 91% yield) as a yellow-orange solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.78 (s, 1H), 8.25-7.94 (m, 7H), 7.76 (d, J = 15.4 Hz, 1H), 7.22 (d, J = 7.5 Hz, 1H), 7.09 (d, J = 8.8 Hz, 2H), 7.03 (t, J = 7.7 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.65 (t, J = 7.5 Hz, 1H), 4.97 (bs, 2H), 3.88-3.70 (m ,4H), 3.48-3.30 (m, 4H).

Table 5



XXIV

Cmpd	Ar	NAME	Characterization	Scheme
XXIVb		*N*-(2-Amino-phenyl)-4-[3-(2-nitro-phenyl)-3-oxo-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (s, 1H), 8.22 (d, J = 7.6 Hz, 1H), 8.03-7.76 (m, 7H), 7.51-7.39 (m, 2H), 7.16 (d, J = 7.6 Hz, 1H), 6.97 (t, J = 7.6 Hz, 1H), 6.78 (d, J = 7.6 Hz, 1H), 6.59 (t, J = 7.3 Hz, 1H), 4.92 (bs, 2H).	8
XXIVc		*N*-(2-Amino-phenyl)-4-[3-(4-methoxy-phenyl)-3-oxo-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.75 (bs, 1H), 8.21 (d, J = 8.8 Hz, 2H), 8.11-8.01 (m, 5H), 7.77 (d, J = 15.4 Hz, 1H), 7.18 (d, J = 7.3 Hz, 1H), 7.11 (d, J = 8.8 Hz, 2H), 6.99 (dd, J = 7.7, 7.7 Hz, 1H), 6.79 (d, J = 7.3 Hz, 1H), 6.61 (dd, J = 7.3, 7.3 Hz, 1H), 4.94 (bs, 2H), 3.88 (s, 3H).	8
XXIVd		*N*-(2-Amino-phenyl)-4-[3-(3-methoxy-phenyl)-3-oxo-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.75 (bs, 1H), 8.08-8.03 (m, 5H), 7.83-7.78 (m, 2H), 7.65 (bs, 1H), 7.51 (dd, J = 7.7, 7.7 Hz, 1H), 7.27 (dd, J = 8.4, 2.3 Hz, 1H), 7.17 (d, J = 8.1Hz, 1H), 6.98 (dd, J = 7.3, 7.3 Hz, 1H), 6.79 (d, J = 7.3 Hz, 1H), 6.60 (dd, J = 7.3, 7.3 Hz, 1H), 4.93 (bs, 2H), 3.87 (s, 3H).	8
XXIVe		*N*-(2-Amino-phenyl)-4-[3-(2-methoxy-phenyl)-3-oxo-propenyl]-benzamide	**^{1}H NMR (300 MHz, DMSO-d_6) δ(ppm) :** 9.73 (bs, 1H), 8.03 (d, J = 8.1 Hz, 2H), 7.88 (d, J = 8.1 Hz, 2H), 7.61-7.50 (m, 4H), 7.22 (d, J = 8.1Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H), 7.08 (dd, J = 7.3, 7.3 Hz, 1H), 6.98 (dd, J = 7.7, 7.7 Hz, 1H), 6.78 (d, J = 7.3 Hz, 1H), 6.60 (dd, J = 7.7, 7.7 Hz, 1H), 4.93 (bs, 2H), 3.89 (s, 3H).	8

Example 43

*N-(2-Amino-phenyl)-4-(3-oxo-3-phenyl-propyl)-benzamide (**XXVIIa**)*

Step 1: 4-(3-Oxo-3-phenyl-propyl)-benzoic acid (**XXVIa**)

[0145] To a stirred solution at room temperature of chalcone **XXIIIa** (1.29 g, 5.13 mmol) in DMF (20 ml) was added phenylsulfonylhydrazine (1.76 g, 10.26 mmol). The reaction mixture was stirred at 110°C for 15 h, cooled and concentrated. The remained oily residue was partitioned between a saturated aqueous solution of NH_4Cl and AcOEt. After separation, the organic layer was dried, partially evaporated and filtered. The filtrate was purified by flash chromatography on silica gel (AcOEt/hexane : 50/50□75/25) to form a material which after a second column purification ($MeOH/CH_2Cl_2$: 5/95) afford the title compound **XXVIa** (400 mg, 1.59 mmol, 31% yield).

Step 2: *N*-(2-Amino-phenyl)-4-(3-oxo-3-phenyl-propyl)-benzamide (**XXVIIa**)

[0146] The title compound **XXVIIa** was obtained from **XXVIa** in one step following the same procedure as **Example 1**, step 2 (Scheme 1). ^1H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.59 (s, 1H); 8.00 (d, J = 7.5 Hz, 2H); 7.90 (d, J = 7.9 Hz, 2H), 7.64 (t, J = 7.5 Hz, 2H), 7.43 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 7.5 Hz, 1H), 6.97 (t, J = 7.0 Hz, 1H), 6.78 (d, J = 7.0 Hz, 1H), 6.59 (t, J = 7.5 Hz, 1H), 4.88 (bs, 2H), 3.44 (t, J = 7.3 Hz, 2H), 3.03 (t, J = 7.3 Hz, 2H).

Example 44

*N-(2-Amino-phenyl)-4-(3-phenyl-propyl)-benzamide (**XXIXa**)*

Step 1: 4-(3-Phenyl-propyl)-benzoic acid (**XXVIIIa**)

[0147] A stirred solution at room temperature of **XXIIIa** (1.34 g, 5.31 mmol) in 25 ml DMA was hydrogenated over 10% Pd/C (600 mg, Degussa type) at 1 atm for 3 h. After removal of the catalyst by filtration through celite pad, the solution was concentrated and the residue was treated with water. After precipitation, the suspension was filtered off, rinsed with H_2O, and dried to afford the title compound **XXVIIIa** (1.13 g, 4.72 mmol, 89% yield).

Step 2: *N*-(2-Amino-phenyl)-4-(3-phenyl-propyl)-benzamide (**XXIXa**)

[0148] The title compound **XXIXa** was obtained from **XXVIIIa** in one step following the same procedure as **Example 1**, step 2 (Scheme 1). ^1H NMR (300 MHz, DMSO-d_6) δ(ppm) : 9.60 (s, 1H); 7.91 (d, J = 7.9 Hz, 2H); 7.34 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 7.23-7.15 (m, 4H), 6.97 (t, J = 7.0 Hz, 1H), 6.78 (d, J = 7.5 Hz, 1H), 6.59 (t, J = 7.3 Hz, 1H), 4.88 (bs, 2H), 2.71-2.59 (m, 4H), 1.92 (m, 2H).

Example 45

N-(2-Amino-phenyl)-4-[3-(1,3-dihydro-isoindol-2-yl)-propenyl]-benzamide (***XXXa***)

Step 1: Methyl 4-trimethylsilanylethynyl-benzoate (**XXXI**)

[0149] A stirred solution at room temperature of methyl 4-bromobenzoate (8.84 g, 41.11 mmol), $Pd(PPh_3)_2Cl_2$ (840 mg, 1.20 mmol) and CuI (455 mg, 2.39 mmol) in anhydrous THF (200 ml) was saturated with nitrogen for 15 min. Then, the solution under nitrogen was cooled down at 0°C, and trimethylsilylacetylene (7.2 ml, 50.91 mmol) and triethylamine (22 ml, 157.8 mmol) were added successively. The reaction mixture was allowed to warm up at room temperature. After 2 h, $Pd(PPh_3)_2Cl_2$ (100 mg) and CuI (80 mg) and trimethylsilylacetylene (0.5 ml) were added again, and the reaction mixture was stirred overnight. Then, the reaction mixture was diluted with AcOEt and successively washed with a saturated aqueous solution of NH_4Cl and brine, dried over $MgSO_4$, filtered and concentrated. The crude residue was then purified by flash chromatography on silica gel (AcOEt/hexane : 5/95□10/90) to afford the title compound **XXXI** (9.05 g, 38.95 mmol, 94% yield) as a yellow sticky solid. ^1H NMR: (400 MHz, $CDCl_3$) δ (ppm) : AB system (δ_A = 7.67, δ_B = 7.22, J_{AB} = 8.5 Hz, 4H), 3.63 (s, 3H), 0.00 (s, 9H).

Step 2: Methyl 4-ethynyl-benzoate (**XXXII**)

[0150] To a stirred solution at 0°C under nitrogen of **XXXI** (9.05 g, 38.95 mmol) in MeOH (280 ml) was added potassium carbonate (1.62 g, 11.72 mmol). After 3 h, the reaction mixture was concentrated and directly purified by flash chromatography on silica gel (CH_2Cl_2 : 100) to afford the title compound **XXXII** (6.16 g, 38.46 mmol, 98% yield) as a pale yellow solid. ^1H NMR: (400 MHz, $CDCl_3$) δ (ppm) : AB system (δ_A = 7.98, δ_B = 7.54, J_{AB} = 8.6 Hz, 4H), 3.93 (s, 3H), 3.24 (s, 1H).

Step 3: β-(4-methoxycarbonyl)-styrylboronic acid (**XXXIII**)

[0151] To a stirred solution at room temperature under nitrogen of **XXXII** (6.16 g, 38.46 mmol) in anhydrous THF (15 ml) was added catecholborane (4.52 ml, 42.80 mmol). The reaction mixture was heated to 70°C for 4 h, and cathecholborane (2 ml) was added again. After 1.5 h, the reaction mixture was allowed to cool down at room temperature, and an aqueous solution of 2N HCl (50 ml) was added and stirred overnight. Then, it was concentrated on the Rotavap, filtered off and the cake was triturated in toluene. After filtration, the intermediate solid was dissolved in THF (50 ml) and an aqueous solution of 2N HCl (150 ml) was added. The resulting suspension was warmed to 40°C for overnight, filtered off, rinsed with water, air-dried and dried under vacuum to afford the title compound **XXXIII** (3.10 g, 15.05 mmol, 39% yield) as an off-white fluffy solid. ^1H NMR: (400 MHz,

DMSO-d_6) δ (ppm) : AB system (δ_A = 7.96, δ_B = 7.63, J_{AB} = 8.4 Hz, 4H), 7.94 (s, 2H), 7.32 (d, J = 18.2 Hz, 1H), 6.30 (d, J = 18.2 Hz, 1H), 3.88 (s, 3H).

Step 4: Methyl 4-[3-(1,3-Dihydro-isoindol-2-yl)-propenyl]-benzoate (**XXXIVa**)

[0152] To a stirred solution pre-heated at 90°C for 15 min under nitrogen of isoindoline (116 mg, 0.97 mmol) and paraformaldehyde (32 mg, 1.07 mmol) in anhydrous 1,4-dioxane (10 ml) was added **XXXIII** (245 mg, 1.17 mmol). After stirring at 90°C for overnight, the reaction mixture was allowed to cool down to room temperature, an aqueous solution of 2N HCl (30 ml) was added and shacked for 30 min. Then, the aqueous mixture was extracted with Et_2O, basified with 2N NaOH (50 ml), and extracted with CH_2Cl_2. The combined dichoromethane layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was then purified by flash chromatography on silica gel ($MeOH/CH_2Cl_2$: 5/95) to afford the title compound **XXXIVa** (135 mg, 0.46 mmol, 48% yield) as an off-white solid. ^1H NMR: (400 MHz, DMSO-d_6) δ (ppm) : AB system (δ_A = 7.93, δ_B = 7.64, J_{AB} = 8.4 Hz, 4H), 7.29-7.17 (m,4H), 6.75 (d, J = 15.8 Hz, 1H), 6.62 (dt, J = 16.0, 6.3 Hz, 1H), 3.94 (s, 4H), 3.88 (s, 3H), 3.55 (dd, J = 6.1, 1.0 Hz, 2H).

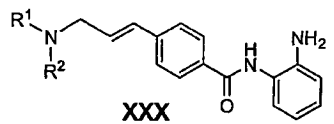
Step 5: *N*-(2-Amino-phenyl)-4-[3-(1,3-dihydro-isoindol-2-yl)-propenyl]-benzamide (**XXXa**)

[0153] The title compound **XXXa** was obtained from **XXXIVa** in two steps following the same procedure as **Example 11**, steps 5 and 6 (Scheme 3). ^1H NMR (400 MHz, DMSO-d_6) δ(ppm) : 9.67 (s, 1H), AB system (δ_A = 7.98, δ_B = 7.63, J_{AB} = 8.3 Hz, 4H), 7.30-7.15 (m,5H), 7.00 (td, J = 7.6, 1.5 Hz, 1H), 6.81 (dd, J = 8.0, 1.4 Hz, 1H), 6.75 (d, J = 15.8 Hz, 1H), 6.66-6.56 (m, 2H), 4.93 (s, 2H), 3.95 (s, 4H), 3.56 (dd, J = 6.2, 0.9 Hz, 2H).

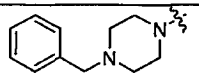
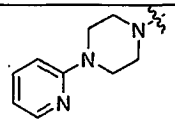
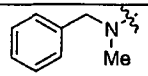
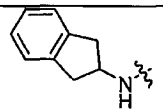
Examples 46-49

[0154] **Examples 46 to 49** (compounds **XXXb-XXXe**) were prepared using the same procedure as described for compound **XXXa** of **Example 45** (Scheme 11).

Table 6



XXX

Cmpd	RX-	NAME	Characterization	Scheme
XXXb		*N*-(2-Amino-phenyl)-4-[3-(4-benzyl-piperazin-1-yl)-propenyl]-benzamide	**^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm) :** 7.84 (d, J = 8.0 Hz, 3H), 7.46 (d, J = 8.0 Hz, 2H), 7.35-7.22 (m, 6H), 7.09 (td, J = 7.6, 1.6 Hz, 1H), 6.88-6.81 (m, 2H), 6.58 (d, J = 15.6 Hz, 1H), 6.41 (dt, J = 15.6, 6.8 Hz, 1H), 3.88 (bs, 2H), 3.57 (s, 2H), 3.24 (d, J = 6.8 Hz, 2H), 2.59 (bs, 8H).	11
XXXc		*N*-(2-Amino-phenyl)-4-[3-(4-pyridin-2-yl-piperazin-1-yl)-propenyl]-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm)** : 9.63 (s, 1H), 8.09-8.08 (m, 1H), 7.93 (d, J = 8.0 Hz, 2H), 8.0 (d, J = 2.5 Hz, 1H), 7.51 (t, J = 7.3 Hz, 1H), 7.14 (d, J = 7.6, 1H), 6.95 (td, J = 7.6, 1.2 Hz, 1H), 6.81 (d, J = 8.6, 1H), 6.76 (dd, J = 8.0, 1.4 Hz, 1H), 6.69-6.60 (m, 2H), 6.58 (td, J = 7.6, 1.3 Hz, 1H), 6.49 (dt, J = 16.0, 6.5 Hz, 1H), 4.89 (s, 2H), 3.48-3.15 (m, 6H), 2.53-2.45 (m, 4H).	11
XXXd		*N*-(2-Amino-phenyl)-4-[3-(benzyl-methyl-amino)-propenyl]-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) :** 9.66 (s, 1H), 7.96 (bd, J = 8.2 Hz, 2H), 7.60 (bd, J = 8.2 Hz, 2H), 7.34 (s, 2H), 7.36 (d, J = 1.2 Hz, 2H), 7.30-7.25 (m, 1H), 7.18 (d, J = 7.0 Hz 1H), 6.99 (td, J = 7.6, 1.5 Hz, 1H), 6.76 (dd, J = 7.9, 1.5 Hz, 1H), 6.68 (d, J = 15.8, 1H), 6.62 (td, J = 7.5, 1.4 Hz, 1H), 6.54 (dt, J = 16.2, 6.5 Hz, 1H), 4.92 (bs, 2H), 3.57 (s, 2H), 3.22 (d, J= 6.3 Hz, 2H), 2.20 (s, 3H).	11
XXXe		*N*-(2-Amino-phenyl)-4-[3-(indan-2-ylamino)-propenyl]-benzamide	**^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm) :** 9.71 (s, 1H), 8.02 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.4 Hz, 2H), 7.32-7.22 (m, 4H), 7.18 (d, J = 7.6 Hz, 1H), 7.00 (t, J = 8.1 Hz, 1H), 6.87 (d, J = 16.0 Hz, 1H), 6.80 (d, J = 9.2 Hz, 1H), 6.63 (t, J = 7.7 Hz, 1H), 6.50 (dt, J = 15.8, 6.7 Hz, 1H), 4.93 (bs, 2H), 4.13 (m, 1H), 3.87 (d, J = 7.6 Hz, 2H), 3.37-3.32 (m, 2H), 3.14 (d, J = 7.6 Hz, 2H).	11

Example 50

Inhibition of Histone Deacetylase Enzymatic Activity

1. Human HDAC-1: Assay 1

[0155] HDAC inhibitors were screened against a cloned recombinant human HDAC-1 enzyme expressed and purified from a Baculovirus insect cell expression system. For deacetylase assays, 20,000 cpm of the [^{3}H]-metabolically labeled acetylated histone substrate (M. Yoshida *et al.*, *J. Biol. Chem.* **265(28)**: 17174-17179 (1990)) was incubated with 30 μg of the cloned recombinant hHDAC-1 for 10 minutes at 37 °C. The reaction was stopped by adding acetic acid (0.04 M, final concentration) and HCl (250 mM, final concentration). The mixture was extracted with ethyl acetate and the released [^{3}H]-acetic acid was quantified by scintillation counting. For inhibition studies, the enzyme was preincubated with compounds at 4 °C for 30 minutes prior to initiation of the enzymatic assay. IC_{50} values for HDAC enzyme inhibitors were determined by performing dose response curves with individual compounds and determining the concentration of inhibitor producing fifty percent of the maximal inhibition. IC_{50} values for representative compounds assayed using this procedure are presented in the third column of Tables 7-10 (excepting bracketed data).

2. Human HDAC-1: Assay 2

[0156] In the alternative, the following protocol was used to assay the compounds of the invention. In the assay, the buffer used was 25mM HEPES, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$ and the substrate was Boc-Lys(Ac)-AMC in a 50mM stock solution in DMSO. The enzyme stock solution was 4.08 μg/mL in buffer.

[0157] The compounds were pre-incubated (2μl in DMSO diluted to 13 μl in buffer for transfer to assay plate) with enzyme (20μl of 4.08μg/ml) for 10 minutes at room temperature (35μl pre-incubation volume). The mixture was pre-incubated for 5 minutes at room temperature. The reaction was started by bringing the temperature to 37°C and adding 16 μl substrate. Total reaction volume was 50μl. The reaction was stopped after 20 minutes by addition of 50μl developer, prepared as directed by Biomol (Fluor-de-Lys developer, Cat. # KI-105). A plate was incubated in the dark for 10 minutes at room temperature before reading (λ_{Ex}=360nm, λ_{Em}=470nm, Cutoff filter at 435nm).

[0158] IC_{50} values for representative compounds assayed using this procedure are presented in the third column of Table 9 (bracketed [ ] data).

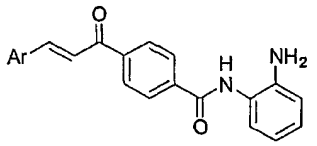
3. MTT Assay

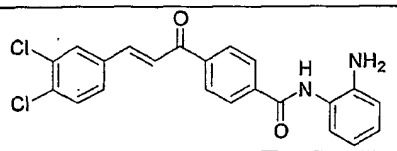
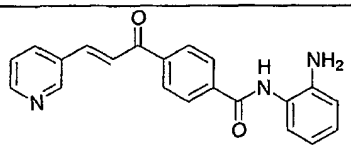
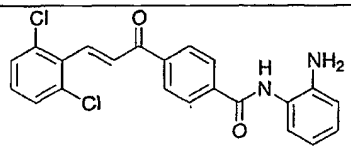
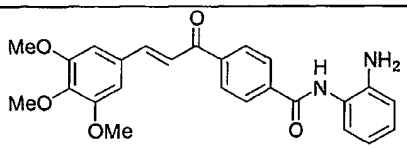
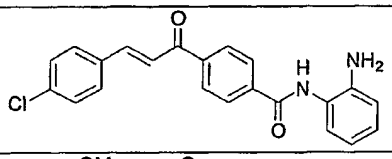
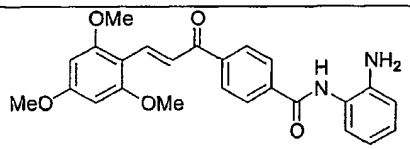
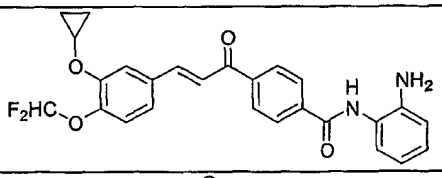
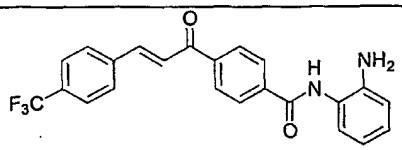
[0159] HCT116 cells (2000/well) were plated into 96-well tissue culture plates one day before compound treatment. Compounds at various concentrations were added to the cells. The cells were incubated for 72 hours at 37°C in 5% CO_2 incubator. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide, Sigma) was added at a final concentration of 0.5 mg/ml and incubated with the cells for 4 hours before one volume of solubilization buffer (50% N,N-dimethylformamide, 20% SDS, pH 4.7) was added onto the cultured cells. After overnight incubation, solubilized dye was quantified by colorimetric reading at 570 nM using a reference at 630 nM using an MR700 plate reader (Dynatech Laboratories Inc.). OD values were converted to cell numbers according to a standard growth curve of the relevant cell line. The concentration which reduces cell numbers to 50% of that of solvent treated cells is determined as MTT IC_{50}. IC_{50} values for representative compounds are presented in the fourth column of Tables 7-10.

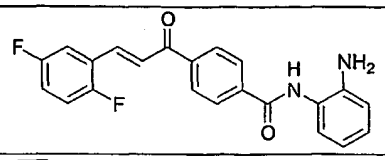
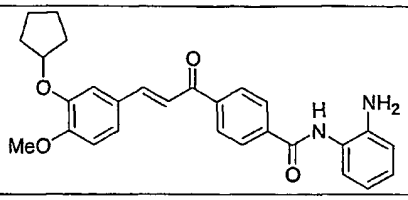
4. Histone H4 acetylation in whole cells by immunoblots

[0160] T24 human bladder cancer cells growing in culture were incubated with HDAC inhibitors for 16 h. Histones were extracted from the cells after the culture period as described by M. Yoshida *et al.* (*J. Biol. Chem.* **265(28)**: 17174-17179 (1990)). 20 g of total histone protein was loaded onto SDS/PAGE and transferred to nitrocellulose membranes. Membranes were probed with polyclonal antibodies specific for acetylated histone H-4 (Upstate Biotech Inc.), followed by horse radish peroxidase conjugated secondary antibodies (Sigma). Enhanced Chemiluminescence (ECL) (Amersham) detection was performed using Kodak films (Eastman Kodak). Acetylated H-4 signal was quantified by densitometry. Representative data are presented in the fifth column of Table 7-10. Data are presented as the concentration effective for reducing the acetylated H-4 signal by 50% (EC_{50}).

Table 7

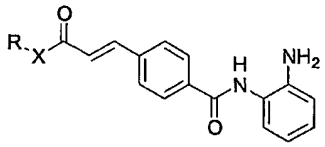


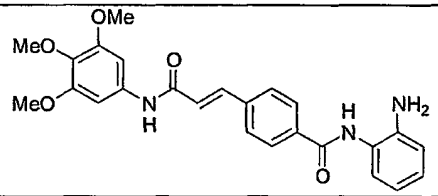
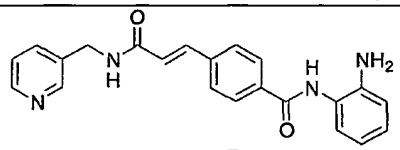
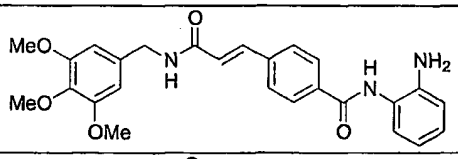
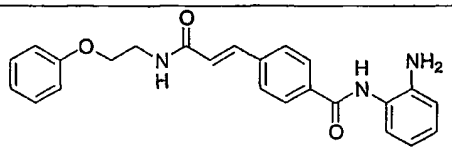
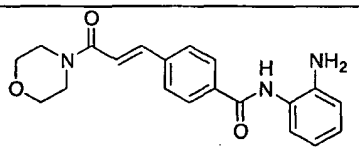
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
Ia		5	1	na
Ib		2	0.9	5
Ic		3	0.4	9999
Id		3	0.6	3
Ie		6	9	na
If		4	5	na
Ig		3	0.7	9999
Ih		5	1	5

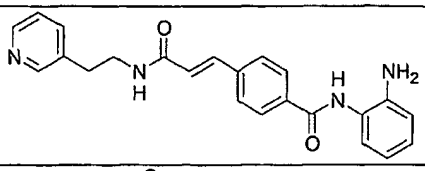
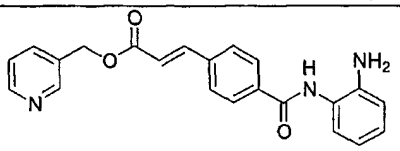
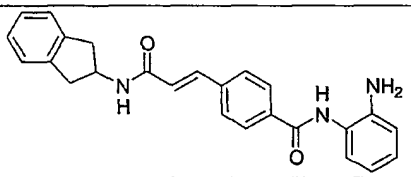
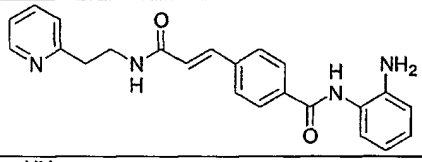
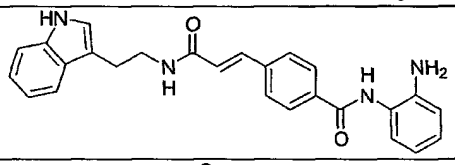
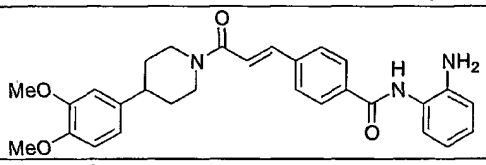
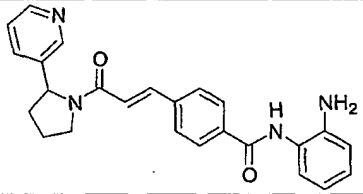
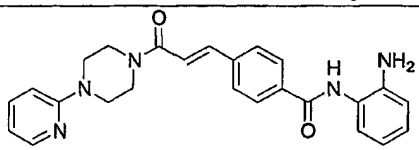
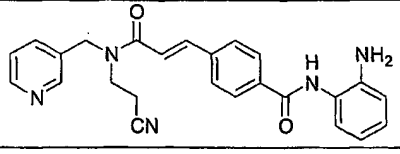
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
Ii		3	1	3
Ij		4	0.7	5

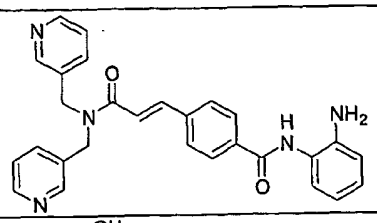
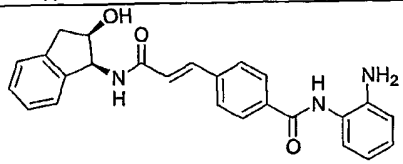
(na=non available, 9999 = >25 mM)

Table 8



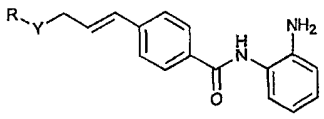
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
Va		5	3	na
Vb		3	0.07	1
Vc		3	0.9	2
Vd		1	0.4	1
Ve		20	6	na

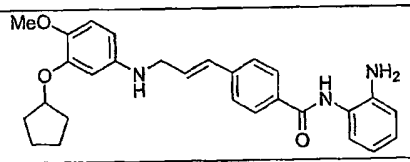
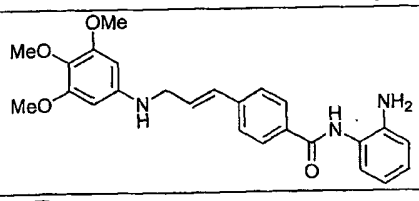
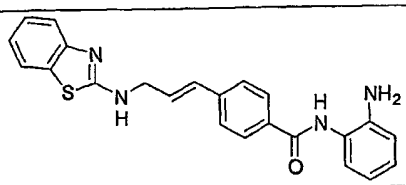
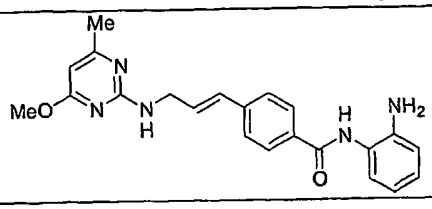
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
Vf		5	0.3	1
Vg		10	5	na
Vh		3	1	10
Vi		4	1	3
Vj		1	0.3	1
Vk		13	2	na
Vl		12	4	na
Vm		11	6	na
Vn		7	3	na

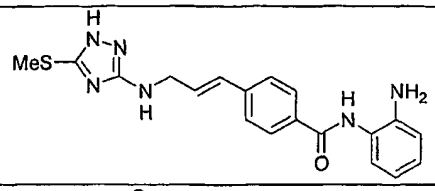
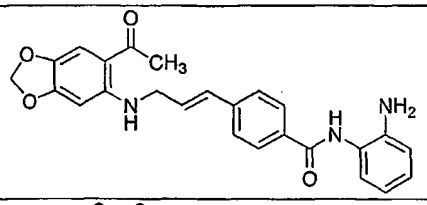
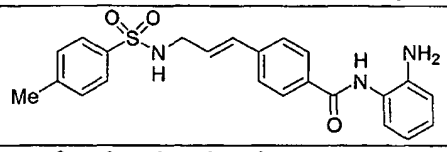
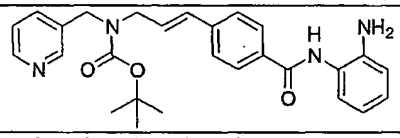
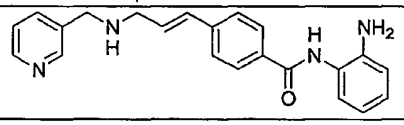
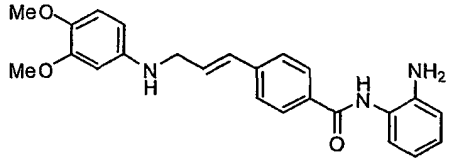
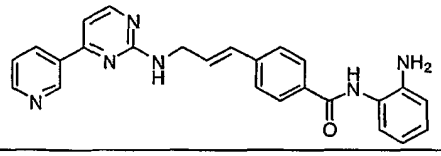
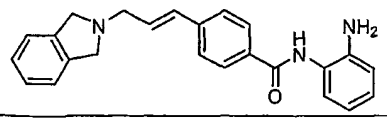
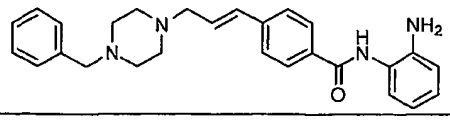
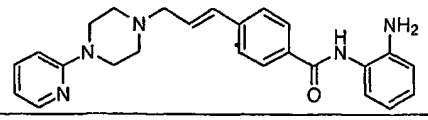
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
Vo		15	4	na
Vp		13	2	na

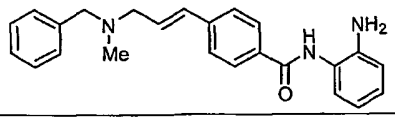
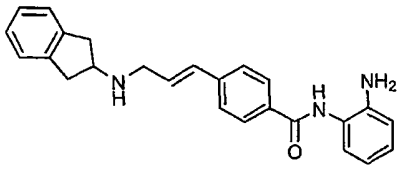
(na=non available, 9999 = >25 mM)

Table 9



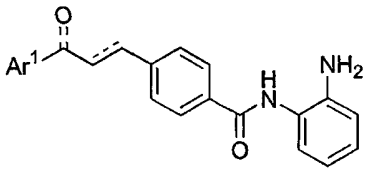
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
XVa		2	1	1
XVb		4	0.1	3
XVc		0.8	1	<1
XVd		4	0.3	3

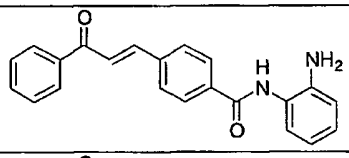
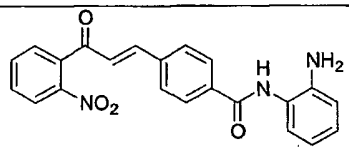
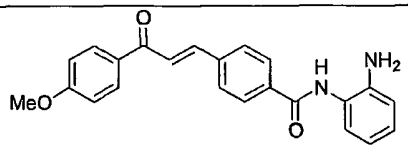
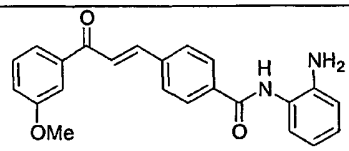
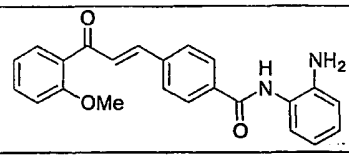
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
XVe		2	0.5	1
XVf		3	1	2
XVg		12	5	na
XXIIh		3	3	na
XVh		4	0.7	2
XVi		1	1	4
XVj		2	0.2	<1
XXXa		[0.26]	1	4
XXXb		4	1	2
XXXc		[0.82]	0.5	2

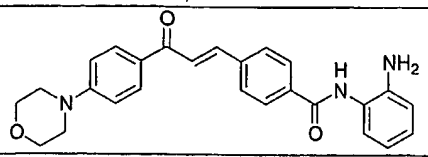
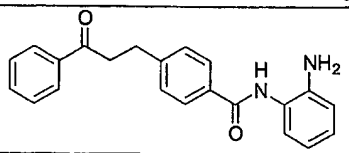
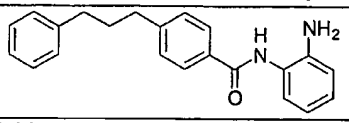
Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
XXXd		[0.17]	2	na
XXXe		[0.79]	2	na

(na=non available, 9999 = >25 mM)

Table 10



Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
XXIVa		4	0.4	3
XXIVb		8	0.1	3
XXIVc		13	0.6	5
XXIVd		4	0.3	>5
XXIVe		3	0.8	5

Cmpd	Structure	Human HDAC-1 IC_{50} (μM)	MTT(HCT116) IC_{50} (μM)	H4 Ac (T24) EC_{50} (μM)
XXIVf		10	0.3	3
XXVIIa		2	0.6	2
XXIXa		2	0.6	na

(na=non available, 9999 = >25 mM)

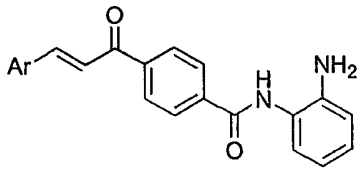
Example 51

Antineoplastic Effects of Histone Deacetylase Inhibitors on Human Tumor Xenografts In Vivo

[0161] Eight to ten week old female CD1 nude mice (Taconic Labs, Great Barrington, NY) were injected subcutaneously in the flank area with 2 x 10^6 preconditioned HCT116 human colorectal carcinoma cells. Preconditioning of these cells was done by a minimum of three consecutive tumor transplantations in the same strain of nude mice. Subsequently, tumor fragments of approximately 30 mgs were excised and implanted subcutaneously in mice, in the left flank area, under Forene anesthesia (Abbott Labs, Geneva, Switzerland). When the tumors reached a mean volume of 100 mm^3, the mice were treated intravenously, subcutaneously, or intraperitoneally by daily injection, with a solution of the histone deacetylase inhibitor in an appropriate vehicle, such as PBS, DMSO/water, or Tween 80/water, at a starting dose of 10 mg/kg. The optimal dose of the HDAC inhibitor was established by dose response experiments according to standard protocols. Tumor volume was calculated every second day post infusion according to standard methods (*e.g.*, Meyer *et al.*, *Int. J. Cancer* **43**: 851-856 (1989)). Treatment with the HDAC inhibitors according to the invention caused a significant reduction in tumor weight and volume relative to controls treated with vehicle only (*i.e.*, no HDAC inhibitor); a subset of these compounds showed toxicity. The results for compound XVj as an example are displayed in Figure 1.

We claim:

1. A compound of the following formula:



or pharmaceutically acceptable salt thereof, wherein

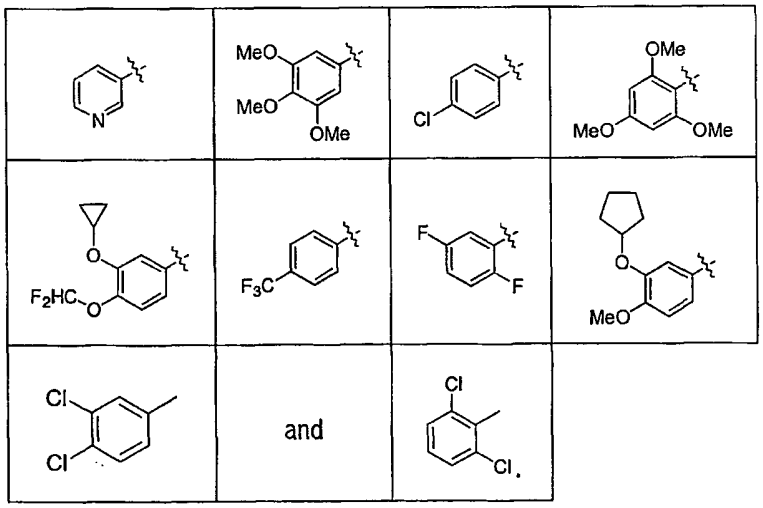
Ar is aryl or heteroaryl, each of which is optionally substituted with from 1 to 3 substituents.

2. The compound of claim 1 wherein Ar is aryl or pyridinyl.

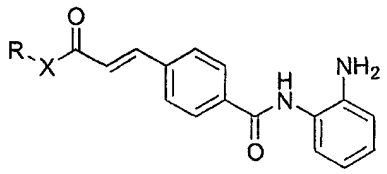
3. The compound of claim 1 wherein Ar is phenyl.

4. The compound of claim 1 wherein Ar is substituted with 1-3 substituents selected from the group consisting of halo, C_1-C_6-hydrocarbyl optionally substituted with halo, C_1-C_6-hydrocarbyloxy optionally substituted with halo.

5. The compound of claim 1 wherein Ar is selected from one of the following:



6. A compound of the following formula:



or pharmaceutically acceptable salt thereof, wherein

X is -N(R^1)-, -O-, or -S-; or X is a nitrogen-containing heterocyclyl in which a nitrogen is covalently bound to the adjacent carbonyl in structure V and is optionally substituted with from 1 to 3 substituents; and

R and R^1 independently are -H, or optionally substituted a) C_1-C_6-hydrocarbyl or b) R^2-L-, wherein R^2 is aryl or heteroaryl, L is C_0-C_6-hydrocarbyl-L^1-C_0-C_6-hydrocarbyl, and L^1 is a covalent bond, -O-, -S-, or -NH-.

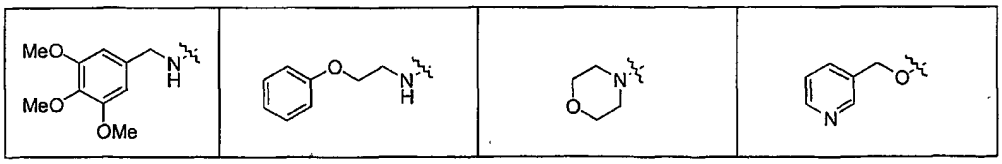
7. The compound according to claim 6 wherein X is -NH-, -O-, morphilin-4-yl, piperidin-1-yl, piperizin-1-yl, or pyrrolidin-1-yl.

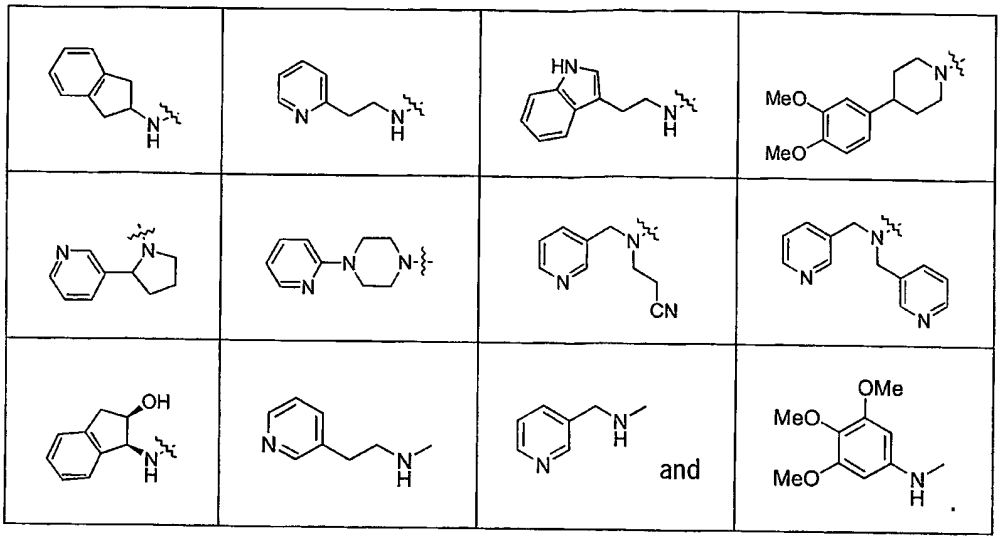
8. The compound according to claim 6 wherein X is -N(R^1)- wherein R^1 is optionally substituted methyl or ethyl.

9. The compound according to claim 6 wherein X is -N(R^1)- wherein R^1 is cyanoethyl or pyridinylmethyl.

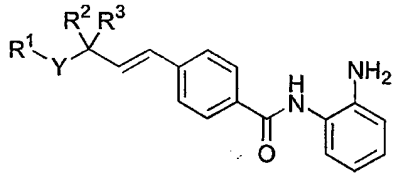
10. The compound according to claim 6 wherein X is -N(R^1)- wherein R is R^2-L- wherein R^2 is phenyl, pyridinyl, indyl, or indolyl and L is a covalent bond, methyl, ethyl, or oxyethyl.

11. The compound according to claim 6 wherein the combination of R-X- is selected from the following:





12. In a third aspect, the invention comprises compounds of the following formula:



or a pharmaceutically acceptable salt thereof, wherein

Y is -N(R^4)-, -O-, -S-, -N(R^4)SO_2-, - SO_2-N(R^4) -, -SO_2-, -N(R^4)-C(O)-, -C(O)-N(R^4)-, -NHC(O)NH-, -N(R^4)C(O)O-, -OC(O)N(R^4)-, or a covalent bond, and

R^1, R^2, and R^3 independently are -H or R^a-C_0-C_6-hydrocarbyl wherein R^a is -H or R^a is aryl or heteroaryl, each of which is optionally substituted with from 1 to 3 substituents.

R^4 is -H, -C(O)-R^b, -C(O)O-R^b, -C(O)NH-R^b ,or R^c-C_0-C_6-hydrocarbyl wherein

R^b is -H or -C_1-C_6-hydrocarbyl, and

R^c is -H, or aryl or heteroaryl each of which is optionally substituted with from 1 to 3 substituents.

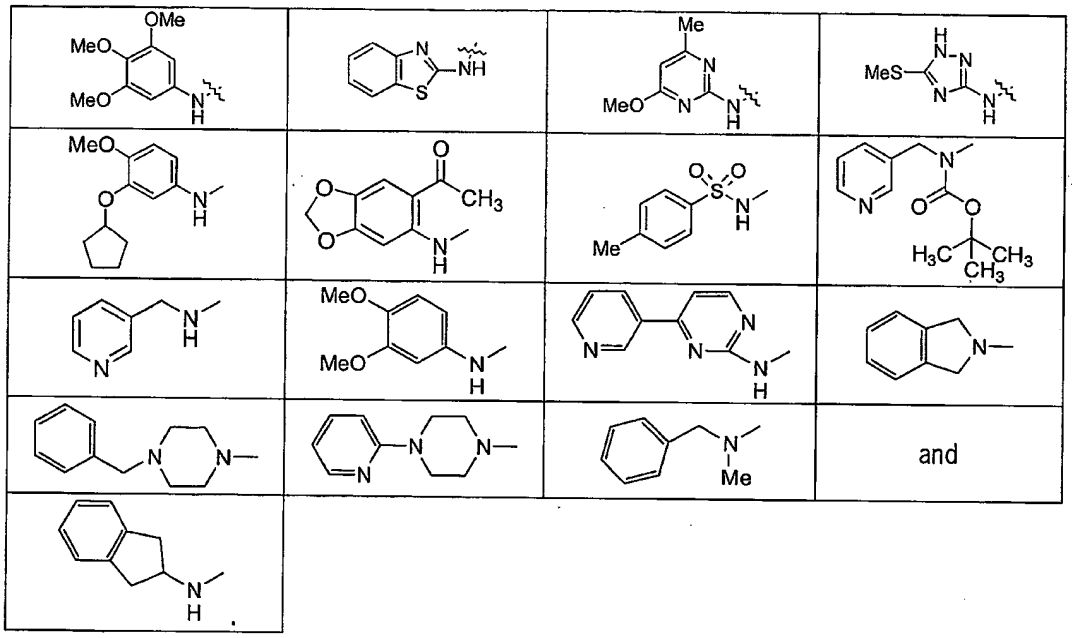
13. The compound according to claim 12 wherein R^2 and R^3 are both -H.

14. The compound according to claim 12 wherein Y is -NH-, -SO_2-NH-, or -N(R^4)- wherein R^4 is -C(O)O-C_1-C_6-hydrocarbyl.

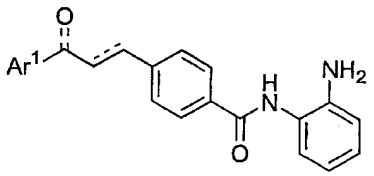
15. The compound according to claim 12 wherein R^1 is aryl, benzothiazolyl, pyrimidinyl, triazolyl, benzodioxolenyl, or pyridinyl, each of which is optionally substituted with from 1 to 3 substituents.

16. The compound according to claim 15 wherein R^1 is substituted with from 1-3 substituents independently selected from C1-C_6-hydrocarbyl, C_1-C_6-hydrocarbyloxy, halo, methylthio, and acetyl.

17. The compound according to claim 12 selected from the following:



18. A compound of formula:

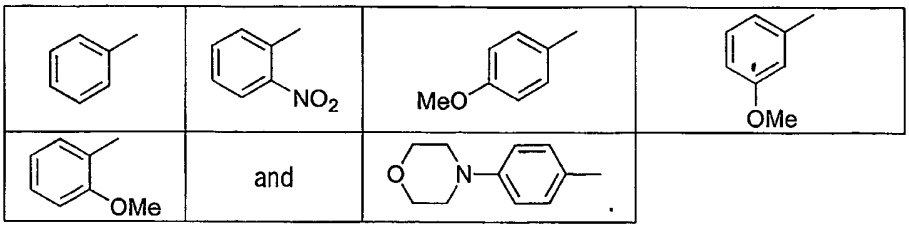


or a pharmaceutically acceptable salt thereof, wherein Ar^1 is aryl or heteroaryl optionally substituted with from 1-3 substituents independently selected from -NO_2, CH_3O-, and morpholinyl (*e.g.*, morpholin-4-yl).

19. The compound according to claim 18 wherein Ar^1 is aryl optionally substituted with from 1-3 substituents independently selected from -NO_2, CH_3O-, and morpholinyl (*e.g.*, morpholin-4-yl).

20. The compound according to claim 18 wherein Ar^1 is phenyl optionally substituted with from 1-3 substituents independently selected from -NO_2, CH_3O-, and morpholinyl (e.g., morpholin-4-yl).

21. The compound according to claim 18 selected from:



22. A composition comprising a compound according to one claims 1 - 21 and a pharmaceutically acceptable carrier, excipient, or diluent.

23. A method of inhibiting histone deacetylase in a cell, comprising contacting a cell in which inhibition of histone deacetylase is desired with an inhibitor of histone deacetylase according to one of paragraphs 1 - 21.

24. A method of treating a mammal suffering from a cell proliferative disease or condition a therapeutically effective amount of a composition according to claim 22.

25. The method according to claim 24 wherein the mammal is a human.

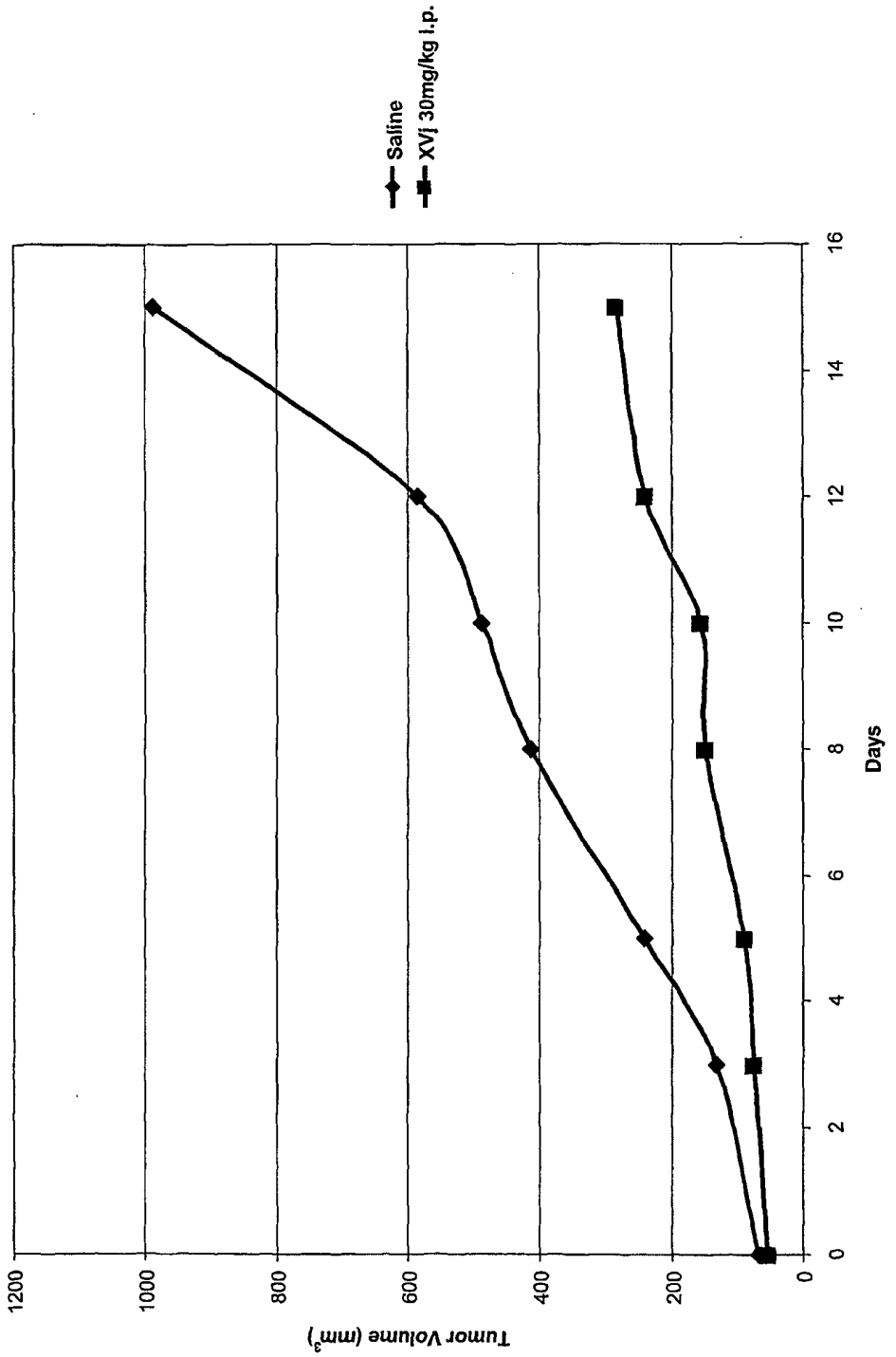

Saline
XVj 30mg/kg i.p.
Tumor Volume (mm³)
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
Days

Figure 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01557

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C235/84 C07D295/18 C07C237/40 C07D295/14 C07C233/80
C07D213/50 C07D213/40 C07D213/30 C07D209/16 C07D211/22
C07D401/04 C07D213/74 C07D277/82 C07D239/46 C07D249/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 847 992 A (MITSUI CHEMICALS INC) 17 June 1998 (1998-06-17) cited in the application claim 1 examples	1-25
Y	WEIDLE U H ET AL: "INHIBITION OF HISTONE DEACETYLASES: A NEW STRATEGY TO TARGET EPIGENETIC MODIFICATIONS FOR ANTICANCER TREATMENT" ANTICANCER RESEARCH, HELENIC ANTICANCER INSTITUTE, ATHENS,, GR, vol. 20, May 2000 (2000-05), pages 1471-1485, XP001098720 ISSN: 0250-7005 page 1481, column 1, first paragraph	1-25

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2004	25/03/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fitz, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01557

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D317/66 C07D213/38 C07D209/44 C07C311/19 A61K31/165
A61K31/40 A61K31/435 A61K31/495 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2004	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Fitz, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/01557

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 23-25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 03/01557

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		JP 3354090 B2	09-12-2002
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